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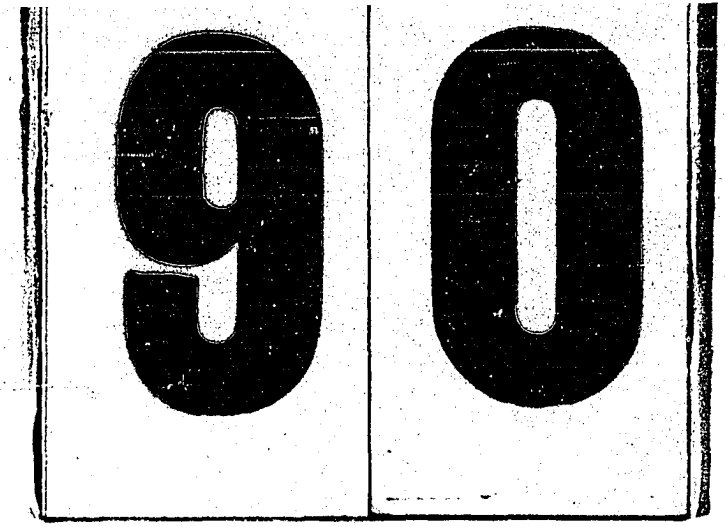
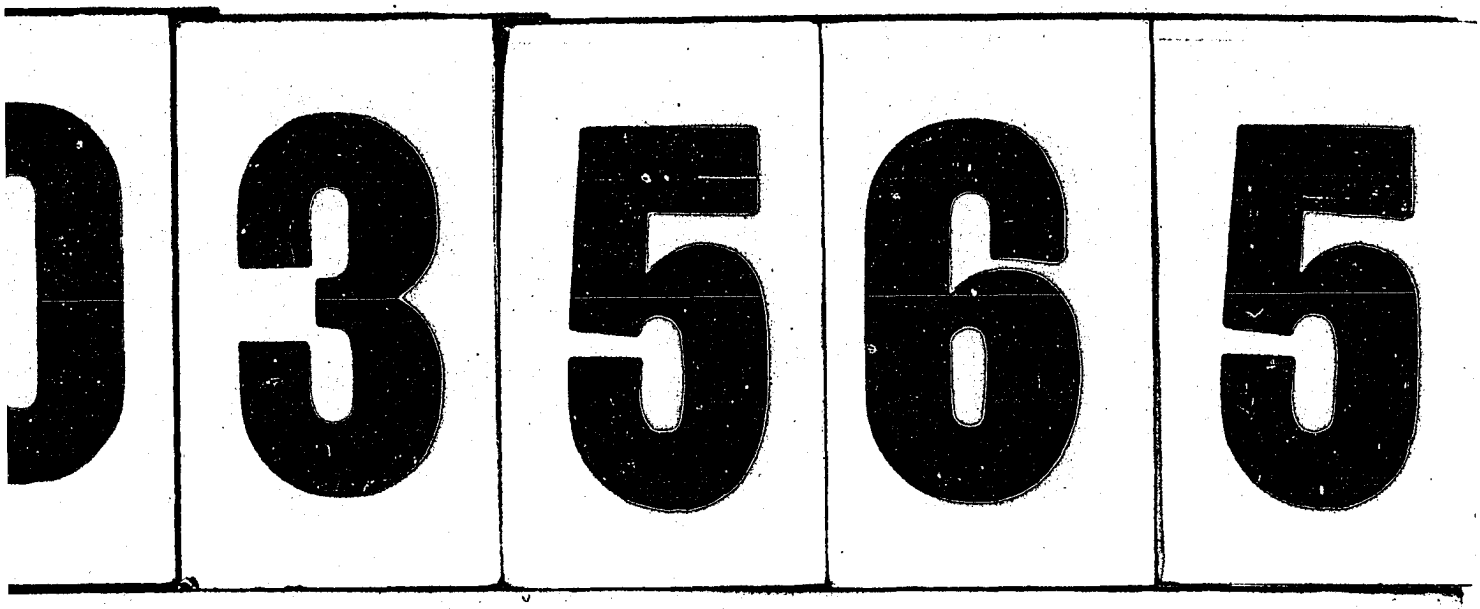
Factors influencing the enhanced microbial degradation of pesticides in soil

L. Somasundaram
Iowa State University





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Somasundaram, L., Ph.D.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

Factors influencing the enhanced microbial degradation of pesticides in soil

by

L. Somasundaram

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GENERAL INTRODUCTION

Degradation of pesticides in soil

Crop protection with chemicals often involves application of pesticides to soil, and in situations where a pesticide is applied on a surface other than soil, a portion of the applied dose usually reaches the soil. The continued dependence on pesticides in global agriculture and the growing concern with environmental pollution by pesticides, reflect the need for better understanding of the fate of pesticides, their metabolites, and impurities in soil.

Soil-applied pesticides dissipate from their site of application through a number of processes such as leaching, volatilization, adsorption, biological and chemical degradation. A growing body of literature has shown that microbial metabolism represents a major route of pesticide degradation in soil. In microbial catabolism, the ultimate objective of the microorganism is to make use of the pesticide or pesticide metabolite as an energy (Sethunathan and Yoshida, 1973) or nutrient (Karns et al., 1986) source. Some microorganisms metabolize pesticides only in the presence of other energy sources and do not derive any benefit from the pesticides; this process is known as cometabolism or incidental metabolism.

Enhanced microbial degradation

The use of chlorinated hydrocarbon and cyclodiene pesticides proved environmentally unacceptable because of their prolonged persistence in soil and in other biological systems. Since the beginning of the 1970's, more biodegradable organophosphorus and carbamate pesticides were

employed as replacements for these recalcitrant pesticides used in crop protection. In the case of biodegradable pesticides, the pesticide should persist at the target site sufficiently long to provide adequate pest control. Since the mid 1970's several researchers have reported inadequate control of target pests by some soil-applied pesticides because of the dramatic decrease in their persistence (Rahman et al., 1979; Felsot et al., 1981).

The failure of these pesticides has been attributed to enhanced microbial degradation, a phenomenon in which adapted soil microorganisms make use of the pesticide or pesticide metabolite as an energy or nutrient source, resulting in decreased persistence of the pesticide and inadequate control of target pests. Although enhanced degradation was first observed 40 years ago for the phenoxyalkanoic acid herbicide 2,4-D (Audus, 1949), the consequences of its importance in crop protection were realized only in the 1980's after the widespread failures of soil-applied pesticides in the Midwestern states of the U.S. Available literature on the susceptibility of EPTC (Rahman et al., 1979), butylate and vernolate (Rudyanski et al., 1987), carbofuran (Felsot et al., 1981) and isofenphos (Abou-Assaf et al., 1987; Racke and Coats, 1987) to enhanced degradation indicates the magnitude of this problem as a potentially serious disruption to the pesticide-based management strategy in crop protection. Similar to the development of resistant strains of pests and the toxicity to non-target organisms, enhanced microbial degradation seems to be yet another problem of using pesticides in agriculture.

Although the current study is focused on corn rootworm insecticides, enhanced degradation is not unique to corn agriculture. It has been reported in several other ecosystems, such as rice (Sethunathan, 1971), vegetables (Walker et al., 1986; Harris et al., 1988), sorghum (Wilde and Mize, 1984), and turf grass (Niemczyk and Chapman, 1987). The susceptibility of promacyl, an acaricide used to control the cattle tick *Boophilus microplus*, to enhanced degradation in cattle dips also has been recently reported (McDougall, 1988). Because of poor host protection observed in all these systems, there is considerable interest in the scientific community to investigate the enhanced microbial degradation of pesticides. Reviews focusing exclusively on the enhanced microbial degradation of insecticides (Felsot, 1989), herbicides (Roeth, 1986), and fungicides (Walker and Suett, 1986) have summarized the research developments in this area, and the American Chemical Society is sponsoring a symposium on the advances in enhanced microbial degradation research in September, 1989.

Factors influencing enhanced microbial degradation

Research to date has largely focused on confirming the susceptibility of pesticides to enhanced degradation. This phenomenon is a soil-pesticide-microbe interaction, influenced by all three factors. In addition to these primary factors, management practices influencing these factors may also affect the degradation process.

The influence of soil characteristics, such as pH, moisture, and organic matter, on the fate of pesticides has been documented by several researchers. Soil pH and moisture are the most influential soil

properties affecting the microbial degradation of pesticides. In general, the degradation rate of pesticides is slow in soils with a pH of < 6.0 (Read, 1983; Walker et al., 1986; Walker, 1987). Strong adsorptive bonds between the pesticides and soil colloids, coupled with a low bacterial activity, may contribute to the lack of enhanced degradation in acidic soils. Laboratory and field studies of Gorder et al. (1982) showed faster degradation of carbofuran in moist soils. Ongoing field studies on the enhanced degradation of pesticides at the Johnson farm (Ames, IA), and the works of Chapman et al. (1986) indicate that no enhanced degradation of carbofuran takes place in soils repeatedly treated with carbofuran if the soil moisture is low. In general, soil moisture favors faster degradation because of increases in microbial activity in moist soils. Soil organic matter content limits the availability of pesticide to microorganisms by increasing adsorption (Hayes, 1970) and does not seem to favor microbial catabolism of pesticides (Ogram et al., 1985).

Although actinomycetes have been implicated with enhanced degradation on a few occasions (Williams et al., 1976), bacteria are the most important group of microorganisms involved in this phenomenon. Several researchers have isolated bacterial populations such as *Pseudomonas* sp. (Felsot et al., 1981; Racke and Coats, 1987), and *Arthrobacter* sp. (Tam et al., 1987; Racke and Coats, 1988a) in aggressive soils; the potential of bacteria to make use of the pesticide as an energy (Sethunathan and Yoshida, 1973) or nutrient (Karns et al., 1986) source has been

demonstrated. The specificity of bacteria in utilizing isofenphos but not other organophosphorus insecticides (Racke and Coats, 1988a), and their ability to degrade structurally similar methyl carbamate insecticides (Racke and Coats, 1988b) and thiocarbamate herbicides (Wilson, 1984) has been clearly elucidated. The involvement of plasmids (extrachromosomal pieces of DNA) in the enhanced degradation of pesticides (Mueller et al., 1988), and the microbial ecology of pesticide-degraders is being investigated in detail.

In general, hydrolytic reactions are the most significant ones in the microbial metabolism of pesticides (Matsumura, 1980). Pesticide catabolism by adapted soil microorganisms usually involves an initial hydrolysis, followed by further metabolism and utilization of hydrolysis products as carbon or nutrient sources (Cook et al., 1978; Nelson, 1982; Karns et al., 1986). The presence of p-nitrophenol, a hydrolysis metabolite of parathion, has been reported to induce accelerated degradation of parathion, with a concomitant increase in the population of parathion-hydrolyzing microbes that utilized p-nitrophenol as the energy source for their proliferation (Sudhakar-Barik et al., 1979; Ferris and Lichtenstein, 1980). 1-Naphthol and carbofuran phenol, the hydrolysis metabolites of carbaryl and carbofuran, were not utilized as energy sources by microbes and accumulated to relatively high concentrations in soil. Yet the application of these hydrolysis metabolites accelerated the degradation of carbaryl and carbofuran in flooded rice soil (Rajagopal et al., 1986). Obviously more than one property (i.e., serving as an energy source) of the hydrolysis product is

involved in influencing the fate of parent pesticides. In contrast, pretreatment of soil with carbofuran phenol did not condition the soil for enhanced degradation of carbofuran (Harris et al., 1984). In a recent study by Bean et al. (1988), the degradation of butylate was not affected in soil pretreated with butylate sulfone, but the degradation rates were enhanced by butylate sulfoxide. The role of pesticide metabolites in enhanced degradation is not clearly understood, and the properties that render a pesticide susceptible or resistant to the development of enhanced degradation need to be studied further.

Management practices used in crop production, including tillage and manuring, affect a number of factors such as physical, chemical, and biological characteristics of the soil, microbial activity, and microbial diversity (Salter and Schollenberger, 1939; McCalla, 1967; Johnson and Lowery, 1985). The soil characteristics and microbes present in soil greatly influence the rate of pesticide degradation. A study of four different tillage treatments showed no effect of tillage on the rapid degradation of isofenphos (Abou-Assaf et al., 1987). Increased degradation of permethrin, carbofuran, parathion, and fonofos due to manure applications has been observed by several researchers (Doyle et al., 1981; Lichtenstein et al., 1982; Koeppe and Lichtenstein, 1984). However, little information can be inferred from these studies as they had used abnormally high manure loading rates. Application of inorganic fertilizers such as ammonium sulfate inhibited the breakdown of parathion (Ferris and Lichtenstein, 1980).

Dissertation objectives

The overall objective of this dissertation research was to investigate and understand some of the factors associated with enhanced microbial degradation. The two main goals were: (1) to elucidate the role of pesticide hydrolysis metabolites in the development of enhanced microbial degradation, and to determine whether key properties of metabolites can be used to predict the relative susceptibility of pesticides to enhanced degradation; and (2) to investigate the effect of manure application on the degradation of pesticides in soil. Specific objectives include:

1. To determine the ability of pesticide hydrolysis metabolites to induce enhanced microbial degradation of their respective parent compounds.
2. To assess the mobility/availability of hydrolysis metabolites and their parent compounds in soils.
3. To assay the relative toxicity of hydrolysis metabolites and their parent compounds to bacteria.
4. To estimate the short- and long-term history of hog manuring on the persistence and degradation of pesticides in soil.

Explanation of dissertation format

This dissertation has been completed following an alternate format, with each chapter comprising a paper submitted to an appropriate scientific journal. Chapter I addresses the potential of repeated application of hydrolysis metabolites in the induction or inhibition of enhanced degradation, and has been submitted to the Journal of

Environmental Science and Health, Part B. Chapter II addresses the mobility of hydrolysis metabolites and their parent compounds in six different soils, and has been submitted to the Journal of Agricultural and Food Chemistry. The toxicity of hydrolysis metabolites and their parent compounds to bacteria was determined using the Microtox system and the results of this study are included in Chapter III. This section has been submitted to the Bulletin of Environmental Contamination and Toxicology. Chapter IV addresses the effect of hog manuring on the fate of soil-applied pesticides, and has been published in the Bulletin of Environmental Contamination and Toxicology. Reference sections are included at the end of each chapter. An additional reference section at the end of the dissertation lists sources used in the GENERAL INTRODUCTION and SUMMARY sections.

CHAPTER I. DEGRADATION OF PESTICIDES IN SOIL AS INFLUENCED BY THE PRESENCE OF HYDROLYSIS METABOLITES

ABSTRACT

The degradation of eight pesticides was evaluated in a soil pretreated with their respective hydrolysis metabolites. 2,4-Dichlorophenol, p-nitrophenol, and salicylic acid conditioned the soil for enhanced degradation of 2,4-dichlorophenoxyacetic acid (2,4-D), parathion, and isofenphos, respectively. Repeated application of carbofuran phenol, 2-isopropyl-4-methyl-6-hydroxypyrimidine, methyl phenyl sulfone, thiophenol, isopropyl salicylate, and 2,4,5-trichlorophenol had no effect on the rate of degradation of their parent pesticides. Prior exposure of soil to 3,5,6-trichloro-2-pyridinol resulted in increased persistence of its parent compound, chlorpyrifos. Results indicate that pesticide hydrolysis metabolites can affect the induction or inhibition of enhanced microbial degradation of some soil-applied pesticides.

INTRODUCTION

Enhanced biodegradation of pesticides was first observed in the late 1940's for the phenoxyalkanoic acid herbicide 2,4-D (Audus, 1949). Since then, it remained merely an academic curiosity, and only recently, after the failures of EPTC (Rahman et al., 1979), carbofuran (Felsot et al., 1981), and isofenphos (Abou-Assaf et al., 1986), the consequences of its direct practical importance have become apparent.

Enhanced degradation of a pesticide is a complex interaction, influenced by properties of the soil, the pesticide, and the microbes. Several researchers have studied the soil (Abou-Assaf and Coats, 1987; Walker, 1987) and microbial (Fournier et al., 1981; Racke and Coats, 1987) aspects of this phenomenon, but the pesticide properties that may influence enhanced degradation have not been studied in detail. These properties include nutritive value, microbial toxicity, and availability to soil microorganisms. Because the pesticides are metabolized after application to soil, some of them within a short time, the properties of pesticide metabolites may also influence the degradation process.

In general, hydrolytic reactions are the most significant initial steps in microbial pesticide metabolism (Matsumura, 1980). The ability of some pesticide hydrolysis metabolites to induce enhanced degradation of their parent compounds has been observed in rice soils (Sudhakar-Barik et al., 1979; Rajagopal et al., 1986) and in cranberry bogs (Ferris and Lichtenstein, 1980), which remain flooded during part of the year. Similar studies have not been performed in nonflooded soils, and it is important to know if the presence of hydrolysis metabolites in such soils

will induce enhanced microbial degradation of subsequently applied pesticides. In the current study, we pretreated a soil up to four times with hydrolysis metabolites to evaluate the effect of the metabolites on conditioning the soil for enhanced degradation.

MATERIALS AND METHODS

Chemicals

The following chemicals were obtained from the respective sources: (U-ring-^{14}C)carbofuran and carbofuran phenol, FMC Corp., Princeton, NJ; (2-ring-^{14}C)diazinon and 2-isopropyl-4-methyl-6-hydroxypyrimidine, Ciba-Geigy Co., Greensboro, NC; (2,6-phenyl-^{14}C)chlorpyrifos and 3,5,6-trichloro-2-pyridinol, Dow Chemical Co., Midland, MI; (2,6-phenyl-^{14}C)parathion, (U-ring-^{14}C)2,4-dichlorophenoxyacetic acid and (U-ring-^{14}C)2,4-dichlorophenol, Amersham Corp., Arlington Heights, IL; (U-ring-^{14}C)2,4,5-trichlorophenoxyacetic acid, NEN Research Products, Boston, MA; (U-ring-^{14}C)fonofos and methyl phenyl sulfone, Stauffer Chemical Co., Mountain View, CA; (U-ring-^{14}C)isofenphos, Mobay Chemical Corp., Kansas City, MO; p-nitrophenol, 2,4-dichlorophenol, 2,4,5-trichlorophenol and thiophenol, Aldrich Chemical Company Inc., Milwaukee, WI; sodium salicylate, Fisher Scientific Co., Itasca, IL. Isopropyl salicylate was synthesized by reacting salicylic acid with isopropyl alcohol, as described by Hsin (1984).

Soil

A clay loam soil (30% sand, 42.5% silt, 27.5% clay, 3.4% organic matter, pH 6.5, cation exchange capacity 22.6 meq/100 g) collected from the Johnson farm, Ames, IA, was used in this study. This soil was not treated with any pesticide in the previous five years. The soil was passed through an 8-mesh sieve and stored for no longer than eight months at 4°C until used.

Treatments and soil incubation

There were four treatments for each pesticide studied. One of the treatments received no hydrolysis metabolite and served as the control. The other three treatments received one, three, or four applications of the hydrolysis metabolite. Five-hundred micrograms of the metabolite was dissolved in a small volume of acetone and surface-applied to soil samples (50 g dry weight each) in 8-oz French square bottles at five-day intervals. The control treatment also received the same volume of acetone, and all the treatments were replicated twice. A week after the last pretreatment, all the treatments were surface-treated with ^{14}C-labeled pesticide or hydrolysis product (0.25 mg or 0.60 μci/50 g soil). Soils were moistened to field capacity (1/3-bar soil moisture tension) with distilled water, and glass vials containing 0.1N NaOH were placed in each bottle to trap the $^{14}CO_2$ evolved. The bottles were closed tightly and incubated at 25°C. The CO_2 traps were replaced daily for the first week and on alternate days for the subsequent two weeks and then analyzed for $^{14}CO_2$ by liquid scintillation counting.

Analyses

After a three-week incubation, ^{14}C-pesticide residues in the soil were extracted twice with acetone-methanol (1:1) and once with acetone-methanol-dichloromethane (1:1:1) and partitioned into dichloromethane as described by Lichtenstein et al. (1973). Thin-layer chromatographic and autoradiographic techniques were used to characterize carbofuran (Archer, 1976), diazinon (Sethunathan and Yoshida, 1969), chlorpyrifos (Getzin, 1981), parathion and fonofos (Fuhremann and Lichtenstein, 1980),

isofenphos (Hsin, 1984), 2,4-dichlorophenol (Miller et al., 1988), 2,4-D and 2,4,5-T (McCall et al., 1981), and their respective metabolites. Unextractable, soil-bound [^{14}C]pesticide residues were recovered by combustion to $^{14}CO_2$ in a Packard sample oxidizer.

RESULTS AND DISCUSSION

Prior exposure of soils to dichlorophenol resulted in more than 80% of applied 2,4-D being mineralized to $^{14}CO_2$ within four days, as compared with 6% in soils that received no dichlorophenol (Figure 1). Although there was an increased mineralization four days after incubation in soils not treated with dichlorophenol, the amount of $^{14}CO_2$ evolved and bound residues formed was significantly less than that of other treatments (Table I). The rapid mineralization of 2,4-D within a week of incubation when pretreated with dichlorophenol indicates that the hydrolysis metabolite may stimulate the induction of enhanced degradation of the parent compound. 2,4-D was the first pesticide reported to undergo enhanced degradation (Audus, 1949); since then, several researchers have demonstrated the ability of microbes to rapidly degrade 2,4-D. Pretreatment of soil with vanillic acid and protocatechuic acid, which are structurally similar to 2,4-D, and other organic compounds such as glucose and ethanol stimulated the number of 2,4-D degraders and also resulted in a higher degradation rate (Kunc and Rybarova, 1984). However, the effect of pretreatment of 2,4-dichlorophenol (a major metabolite of 2,4-D) on the degradation of 2,4-D has not been studied.

According to Fournier et al. (1981), the degradation of 2,4-D when applied for the first time is more likely by cometabolism, and the enhanced degradation observed after repeated applications is because of the microbes that catabolize 2,4-D for their benefit. The enhanced degradation of 2,4-D observed in the current study indicates that the

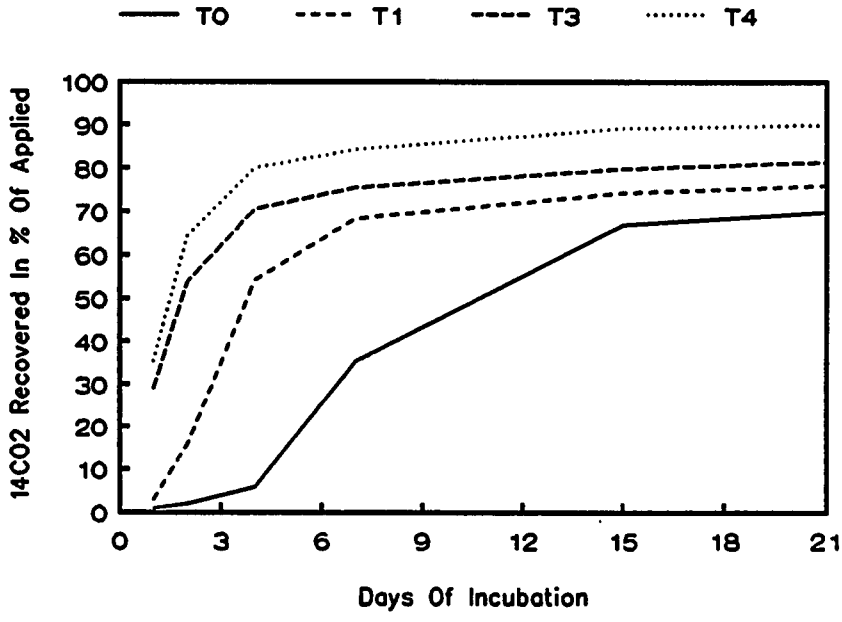

Figure 1. Effect of no pretreatment (T0), one (T1), three (T3), and four (T4) pretreatments of 2,4-dichlorophenol on the evolution of $^{14}CO_2$ from [^{14}C]2,4-D-treated soil

Table I. Effect of pretreatment of hydrolysis metabolites on the degradation of pesticides during a three-week incubation

Fractions	^{14}C recovered, % of applied ^{14}C pesticide			
	Number of pretreatments with hydrolysis metabolites			
	0	1	3	4
	2,4-D (2,4-dichlorophenol)			
2,4-D	0.21[a]	0.22[a]	0.18[a]	0.26[a]
2,4-Dichlorophenol	0.63[a]	0.55[a]	0.40[a]	0.53[a]
Soil-bound	29.46[a]	21.75[b]	18.52[c]	13.18[d]
$^{14}CO_2$	69.88[a]	76.02[b]	81.47[c]	90.01[d]
Others[e]	0.87[a]	0.62[b]	0.47[bc]	0.40[c]
Total	101.05[a]	99.17[a]	101.05[a]	103.06[a]
	2,4-dichlorophenol (2,4-dichlorophenol)			
2,4-Dichlorophenol	5.44[a]	4.95[a]	4.79[a]	3.67[b]
Soil-bound	54.24[a]	55.12[a]	52.55[b]	31.55[c]
$^{14}CO_2$	27.87[a]	26.78[a]	27.57[a]	54.40[b]
Others[e]	2.52[a]	3.50[b]	2.95[ab]	2.03[ab]
Total	90.08[a]	90.35[a]	87.87[b]	91.66[a]

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[e]Includes volatile products other than $^{14}CO_2$ as well as polar, water-soluble products.

hydrolysis metabolite may serve as a favorable substrate for microorganisms involved in the degradation process.

To confirm that the adaptation of microbes to degrade 2,4-D rapidly is mainly because of their ability to use the dichlorophenol, soil was pretreated with 2,4-dichlorophenol and the fate of subsequently applied ^{14}C-2,4-dichlorophenol was studied (Table I). Soil-bound residues and $^{14}CO_2$ were the major products formed in all the treatments. There was little difference in the degradation of dichlorophenol in soil not treated and pretreated one or three times with dichlorophenol. More than 80% of the applied dichlorophenol was recovered as $^{14}CO_2$ and bound residues in all these treatments. However, 54.4% of initially applied dichlorophenol was mineralized to $^{14}CO_2$ in soils treated four times with dichlorophenol as compared with 26.8 to 27.9% in other treatments.

Although the degradation pathway of 2,4-D has been investigated by several researchers, 2,4,5-T has not been studied as completely. Pretreatment of soils with 2,4,5-trichlorophenol had no effect on the fate of subsequently applied ^{14}C-2,4,5-T (Table II). About one-third of the initially applied 2,4,5-T was present as bound residues in all the treatments studied. 2,4,5-Trichloroanisole and 2,4,5-trichlorophenol were the major metabolites formed, and the anisole probably was formed from the phenol through a microbial methylation process. Although considerable anisole is recovered in closed incubations in the laboratory, because of its high volatility significant amounts of anisole may not be present in soil after the field application of 2,4,5-T (McCall et al., 1981).

Table II. Effect of pretreatment of hydrolysis metabolites on the degradation of pesticides during a three-week incubation

Fractions	^{14}C recovered, % of applied ^{14}C pesticide			
	Number of pretreatments with hydrolysis metabolites			
	0	1	3	4
	2,4,5-T (2,4,5-trichlorophenol)			
2,4,5-T	17.84[a]	15.37[b]	15.16[b]	15.75[b]
2,4,5-T-phenol	4.41[a]	4.85[a]	5.30[ab]	6.30[b]
2,4,5-T-anisole	35.91[a]	30.62[a]	36.52[a]	36.21[a]
Soil-bound	30.94[a]	30.21[a]	30.47[a]	29.47[a]
$^{14}CO_2$	9.26[a]	5.58[b]	5.19[b]	5.29[b]
Others[d]	0.74[a]	0.96[a]	0.81[a]	0.63[a]
Total	99.10[a]	87.60[c]	93.47[b]	93.46[b]

[a-c]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[d]Includes volatile products other than $^{14}CO_2$ as well as polar, water-soluble products.

There is no evidence of enhanced degradation of 2,4,5-T, and the microorganisms found to metabolize 2,4,5-T do not use it as a carbon or energy source (Rosenberg and Alexander, 1980). It is therefore not surprising that trichlorophenol did not serve as a microbial substrate. The toxicity of trichlorophenol to microbes (Somasundaram et al., 1989b) and its low availability in soil (Somasundaram et al., 1989a) may contribute to the resistance of 2,4,5-T to the development of enhanced microbial degradation.

Parathion was more persistent in soils that received zero or only one application of p-nitrophenol (Table III). More than 50% of the applied parathion was converted to soil-bound residues and $^{14}CO_2$ in soils pretreated three and four times with p-nitrophenol. Evolution of $^{14}CO_2$ was more pronounced as the number of p-nitrophenol treatments increased. Ferris and Lichtenstein (1980) observed 34% of applied parathion being mineralized in 24 h in soils pretreated with p-nitrophenol as compared with 2% in the control. The potential of p-nitrophenol to serve as a good microbial substrate and the resulting increase in the population of parathion-hydrolyzing microbes in p-nitrophenol treated soils have been reported previously (Sudhakar-Barik and Sethunathan, 1978; Sudhakar-Barik et al., 1979).

The degradation of ^{14}C-ring carbofuran was not altered in soils pretreated with its hydrolysis metabolite, carbofuran phenol (Table III). Nearly two-thirds of the initially applied carbofuran remained in all the treatments. There was little difference in the quantities of

Table III. Effect of pretreatment of hydrolysis metabolites on the degradation of pesticides during a three-week incubation

Fractions	^{14}C recovered, % of applied ^{14}C pesticide			
	Number of pretreatments with hydrolysis metabolites			
	0	1	3	4
	Parathion (p-nitrophenol)			
Parathion	55.01[a]	52.14[b]	40.87[c]	33.84[d]
p-Nitrophenol	0.75[a]	1.78[b]	2.84[c]	3.29[d]
Soil-bound	26.96[a]	27.27[a]	29.85[b]	30.80[b]
$^{14}CO_2$	12.28[a]	13.13[a]	25.57[b]	30.65[c]
Others[e]	1.69[a]	1.91[a]	2.68[a]	3.06[a]
Total	96.70[a]	96.26[a]	101.10[b]	101.65[b]
	Carbofuran (carbofuran phenol)			
Carbofuran	77.01[a]	74.27[b]	77.48[a]	75.20[b]
3-Ketocarbofuran	1.38[a]	1.75[a]	1.67[a]	1.41[a]
Soil-bound	10.84[a]	12.52[b]	13.25[b]	13.06[b]
$^{14}CO_2$	2.75[a]	3.38[a]	3.27[a]	3.08[a]
Others[e]	0.58[a]	1.14[a]	0.58[a]	0.78[a]
Total	92.56[a]	93.07[a]	96.27[a]	93.54[a]

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[e]Includes volatile products other than $^{14}CO_2$ as well as polar, water-soluble products.

carbofuran phenol, soil-bound residues, and $^{14}CO_2$ recovered among the four treatments. Metabolism studies with ^{14}C-carbonyl carbofuran were also conducted, and the results (not shown) were very similar to the ^{14}C-ring data reported in Table III, except for a slight increase in the quantity of $^{14}CO_2$ evolved (11.4 to 13.3%) in all the treatments. Similar observations of the failure of carbofuran phenol to activate soils for enhanced degradation of carbofuran have been reported (Harris et al., 1984). In contrast, in the flooded rice soils of India, carbofuran degradation was more rapid in soils pretreated with its phenolic metabolite (Rajagopal et al., 1986); however, carbofuran phenol accumulated in these soils in large quantities, indicating that it was not utilized as an energy source by microbes. It is not clear as to how carbofuran phenol (without serving as an energy source) stimulated enhanced degradation of carbofuran in that study.

The accumulation of carbofuran phenol in soil (Rajagopal et al., 1986), and its failure to induce enhanced degradation of carbofuran as observed in the current investigation and that of Harris et al. (1984) indicate that the enhanced degradation of carbofuran is not associated with its primary hydrolysis metabolite. The hydrolysis of carbofuran results in the formation of carbofuran phenol and methyl carbamic acid. The latter is not stable in soil and is further converted to methyl amine and CO_2. Karns et al. (1986) isolated a bacterium capable of hydrolyzing the carbamate linkage of carbofuran by using the nitrogen of the N-methyl carbamate side chain as a sole nitrogen source, and rapidly inactivating the compound. These findings suggest that methyl amine, a metabolite of

carbofuran, may be the preferred substrate and responsible for the induction of enhanced degradation of carbofuran.

Prior applications of 2-isopropyl-4-methyl-6-hydroxypyrimidine, the hydrolysis metabolite of diazinon, did not activate the soil to degrade its parent compound rapidly (Table IV). About 37 to 40% of the applied diazinon remained in all the treatments, and there was no major difference in the quantities of metabolites, $^{14}CO_2$, and soil-bound residues recovered. The hydroxypyrimidine metabolite is less toxic to microorganisms (Somasundaram et al., 1989b), and because of its high mobility in soil, it probably is readily available (Somasundaram et al., 1989a) to microorganisms for further mineralization (Sethunathan and Pathak, 1972). On the basis of these characteristics it would seem likely that hydroxypyrimidine might serve as a good microbial substrate and enhance the degradation of diazinon. In this study, however, we found no evidence of enhanced degradation of diazinon in pyrimidine-treated soils. This may be because not all soils contain microbes capable of degrading diazinon.

Of the eight pesticides studied, only for chlorpyrifos did the pretreatment of hydrolysis metabolite result in increased persistence of the parent compound (Table IV). Although more than 70% of applied chlorpyrifos persisted in all the treatments, a slight increase in persistence was observed in trichloropyridinol-treated soils. The mineralization rate was < 10% in all treatments; however, it was about 10-fold higher in soils not exposed to trichloropyridinol. The relatively greater persistence of chlorpyrifos observed in

Table IV. Effect of pretreatment of hydrolysis metabolites on the degradation of pesticides during a three-week incubation

Fractions	^{14}C recovered, % of applied ^{14}C pesticide			
	Number of pretreatments with hydrolysis metabolites			
	0	1	3	4
	Diazinon (hydroxypyrimidine)			
Diazinon	37.90[a]	37.58[a]	39.85[a]	38.59[a]
Hydroxypyrimidine	18.43[a]	18.73[a]	22.00[b]	20.52[ab]
Soil-bound	19.59[a]	18.18[a]	18.16[a]	19.85[a]
$^{14}CO_2$	0.95[a]	1.04[a]	0.77[a]	0.95[a]
Others[e]	12.85[a]	13.74[a]	13.03[a]	14.17[a]
Total	89.73[a]	89.29[a]	93.83[a]	94.08[a]
	Chlorpyrifos (trichloro-2-pyridinol)			
Chlorpyrifos	73.78[a]	79.08[b]	79.61[b]	85.08[c]
3,5,6-T-2-pyridinol	0.78[a]	0.73[a]	0.88[a]	0.73[a]
Soil-bound	6.19[a]	4.72[b]	3.69[c]	2.83[d]
$^{14}CO_2$	5.72[a]	0.76[b]	0.59[b]	0.60[b]
Others[e]	11.79[a]	10.71[a]	13.09[a]	10.40[a]
Total	98.27[a]	96.00[a]	97.87[a]	99.65[a]

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[e]Includes volatile products other than $^{14}CO_2$ as well as polar, water-soluble products.

trichloropyridinol-treated soil may be attributed to the toxicity of pyridinol to microbes. The antibacterial activity of pyridinol has been demonstrated in our laboratory (Racke et al., 1988; Somasundaram et al., 1989b).

Fonofos was relatively persistent in all the four treatments (Table V), and there were no major effects of pretreatment with either of the major hydrolysis metabolites (thiophenol or methyl phenyl sulfone). Thiophenol did exert a small but significant deterrent effect on the degradation of fonofos. There was not much variation among the treatments in terms of $^{14}CO_2$ evolved and bound residues formed.

The ability of microbes to utilize isofenphos as a sole carbon source and the enhanced degradation of isofenphos in field and laboratory situations have been well documented (Abou-Assaf et al., 1986; Racke and Coats, 1987). Previous applications of isopropyl salicylate, a primary hydrolysis metabolite formed in the microbial metabolism of isofenphos, did not enhance the degradation of the parent compound (Table VI). Even after four pretreatments of isopropyl salicylate, 79% of the initially applied ^{14}C-isofenphos remained in soil. There was not much variation in the amounts of isofenphos oxon, bound residues, and $^{14}CO_2$ recovered among the different treatments. However, exposure of soils to salicylic acid, the secondary hydrolysis metabolite of isofenphos, resulted in enhanced degradation of isofenphos (Table VI). At the end of the three-week incubation, only 34% of the applied isofenphos was present in soils treated four times with salicylic acid, as compared with 78% in soil not exposed to this metabolite. The amount of $^{14}CO_2$ evolved and bound

Table V. Effect of pretreatment of hydrolysis metabolites on the degradation of pesticides during a three-week incubation

Fractions	^{14}C recovered, % of applied ^{14}C pesticide			
	Number of pretreatments with hydrolysis metabolites			
	0	1	3	4
	Fonofos (thiophenol)			
Fonofos	46.44[a]	50.42[b]	51.77[b]	52.22[b]
Methyl phenyl sulfone	6.13[a]	7.72[a]	7.35[a]	6.41[a]
Fonofos oxon	0.49[a]	0.48[a]	0.37[b]	0.38[b]
Soil-bound	20.70[a]	18.48[a]	18.24[ab]	15.90[b]
$^{14}CO_2$	11.49[a]	10.76[a]	7.77[b]	9.63[c]
Others[d]	1.28[a]	2.01[a]	2.93[a]	1.23[a]
Total	86.55[a]	89.87[a]	88.44[a]	85.79[a]
	Fonofos (methyl phenyl sulfone)			
Fonofos	50.11[a]	52.64[a]	52.23[a]	51.36[a]
Methyl phenyl sulfone	6.23[a]	8.88[b]	8.45[b]	8.63[b]
Fonofos oxon	0.46[a]	0.38[a]	0.37[a]	0.29[a]
Soil-bound	22.92[a]	19.84[a]	20.62[a]	19.79[a]
$^{14}CO_2$	12.74[a]	11.09[a]	10.00[a]	10.70[a]
Others[d]	1.66[a]	1.13[a]	1.22[a]	0.93[a]
Total	94.13[a]	93.96[a]	92.90[a]	91.71[a]

[a-c]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[d]Includes volatile products other than $^{14}CO_2$ as well as polar, water-soluble products.

Table VI. Effect of pretreatment of hydrolysis metabolites on the degradation of pesticides during a three-week incubation

Fractions	^{14}C recovered, % of applied ^{14}C pesticide			
	Number of pretreatments with hydrolysis metabolites			
	0	1	3	4
	Isofenphos (salicylic acid)			
Isofenphos	78.04[a]	65.30[b]	36.81[c]	33.69[c]
Isofenphos oxon	8.95[a]	7.81[a]	5.35[b]	4.43[b]
Soil-bound	8.75[a]	12.34[b]	21.32[c]	24.90[d]
$^{14}CO_2$	3.91[a]	9.93[b]	34.47[c]	38.72[d]
Others[e]	0.36[a]	0.42[a]	0.26[a]	0.33[a]
Total	100.02[a]	95.81[a]	98.22[a]	102.07[a]
	Isofenphos (isopropyl salicylate)			
Isofenphos	83.69[a]	80.50[b]	79.12[bc]	78.99[c]
Isofenphos oxon	9.48[a]	9.64[a]	9.60[a]	9.29[a]
Soil-bound	6.74[a]	6.56[a]	6.36[a]	5.34[b]
$^{14}CO_2$	4.08[a]	4.15[a]	3.94[a]	3.83[a]
Others[e]	0.24[a]	0.17[a]	0.27[a]	0.18[a]
Total	104.24[a]	101.03[b]	99.30[bc]	97.50[c]

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[e]Includes volatile products other than $^{14}CO_2$ as well as polar, water-soluble products.

residues formed increased in proportion to the number of applications of salicylic acid.

Salicylic acid is less toxic to microbes (Somasundaram et al., 1989b), and some soil bacteria carry degradative plasmids for salicylic acid metabolism (Chakrabarty, 1972). The ability of microbes to metabolize 3,5-dichlorosalicylate, 3,6-dichlorosalicylic acid (Kruger et al., 1989), and 5-chlorosalicylate (Crawford et al., 1979) beneficially has been reported. Racke and Coats (1987) believed that the formation of salicylic acid might be a key factor in the susceptibility of isofenphos to enhanced degradation. The low microbial toxicity (Somasundaram et al., 1989b), relative availability (reflected by its high mobility) (Somasundaram et al., 1989a), and its nutritive value may contribute to the potential for salicylic acid to condition soils for enhanced degradation of isofenphos.

In a recent study by Bean et al. (1988), the degradation of butylate was not affected by soil pretreated with butylate sulfone, but the degradation rates were enhanced by butylate sulfoxide. In the current investigation, salicylic acid was able to condition the soil for enhanced degradation of isofenphos, but isopropyl salicylate did not have any effect. These findings suggest that the microbes involved in enhanced degradation are sensitive to changes associated with the metabolism of the parent pesticide molecule.

Of the 10 hydrolysis metabolites studied, 2,4-dichlorophenol, p-nitrophenol, and salicylic acid conditioned the soil for enhanced degradation of their respective parent pesticides, whereas pretreatments

of 3,5,6-trichloro-2-pyridinol resulted in increased persistence of its parent pesticide. Although the potential of pesticide metabolites to condition soils for enhanced degradation has been previously reported (Sudhakar-Barik et al., 1979; Rajagopal et al., 1986; Bean et al., 1988), this is the first study in which a metabolite has been observed to increase the persistence of its parent compound. The properties of pesticide metabolites such as nutritive value, microbial toxicity, and availability in soil play a critical role in determining whether a metabolite is able to induce or inhibit the enhanced degradative phenomenon. Results obtained from the current study indicate that the secondary hydrolysis metabolites such as salicylic acid and methylamine could play an important role in the environmental fate of their parent compounds. Research to date has largely focused on the fate of pesticides. Future research should also aim at understanding the properties and dynamics of degradation products.

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CHAPTER II. MOBILITY OF PESTICIDES AND THEIR HYDROLYSIS METABOLITES IN SOIL, WITH IMPLICATIONS CONCERNING AVAILABILITY AND DEGRADATION

ABSTRACT

The soil thin-layer chromatography technique was employed to assess the mobility of 21 chemicals (six insecticides, four herbicides, and 11 of their hydrolysis metabolites) in six different soils. The hydrolysis products of the four organophosphorus insecticides studied were significantly more mobile than their parent compounds, but the metabolites of carbamates, s-triazines, and phenoxyalkanoic acids were less mobile than their respective parent compounds. Soil pH significantly affected mobility of all but three chemicals, with increased mobility in soils with higher pHs. Greater levels of organic matter, clay, cation exchange capacity, and moisture content (at 1/3 bar) retarded mobility. Although a significant relationship existed between water solubility and mobility, solubility by itself was not a good predictor of mobility. Structural similarity does not seem to be an indicator of mobility, but chemical structure may affect mobility by influencing adsorption. Mobility plays a key role in the availability of these compounds in soil and enables better understanding of the fate of these compounds in soil.

INTRODUCTION

Movement of a pesticide from its site of application is of significance to the chemicals' bioefficacy, availability, degradation, microbial toxicity, phytotoxicity, and leaching. Although the mobility of herbicides in soil has been studied in detail, little is known about the mobility of herbicide metabolites or other groups of chemicals such as insecticides. Inasmuch as some pesticide metabolites have been associated with potential for bioefficacy (Chapman and Harris, 1980a), enhanced microbial degradation of the parent compound (Rajagopal et al., 1986), phytotoxicity (Kaufman and Blake, 1970), and groundwater contamination (Cohen et al., 1984), more research is needed to understand their movement and fate in soil.

Different techniques such as soil columns (Leistra et al., 1976), lysimeters (Fuhr, 1986), and watershed studies (Norris et al., 1984) have been employed to assess the mobility of chemicals in soil. Helling and Turner (1968) developed a laboratory method to identify potentially mobile compounds in soil. In this technique, known as soil thin-layer chromatography (STLC), thin layers of soil serve as the adsorbent phase and are developed with water by using techniques analogous to conventional thin-layer chromatography. The data obtained from STLC studies correlate well with published observations of field and laboratory investigations on pesticide mobility (Helling and Turner, 1968; Helling, 1971b). In this study, using the STLC technique, we have investigated the mobility of 11 pesticide metabolites along with their respective parent compounds in six different soils. Because hydrolysis

is a significant step in the chemical and microbial degradation of pesticides in soil (Matsumura, 1980), the principal focus of this study was on hydrolytic metabolites.

MATERIALS AND METHODS

Chemicals

Twenty-one different ^{14}C-labeled chemicals (six insecticides, four herbicides, and 11 hydrolysis metabolites) were studied. The compounds obtained from their respective sources were: (2,6-phenyl-^{14}C)-chlorpyrifos and (2,6-phenyl-^{14}C)3,5,6-trichloro-2-pyridinol, Dow Chemical Co., Midland, MI; (2,6-phenyl-^{14}C)parathion, (U-ring-^{14}C)2,4-dichlorophenoxyacetic acid and (U-ring-^{14}C)2,4-dichlorophenol, Amersham Corp., Arlington Heights, IL; (U-ring-^{14}C)2,4,5-trichlorophenoxyacetic acid, NEN Research Products, Boston, MA; (2,6-phenyl-^{14}C)p-nitrophenol, International Chemical & Nuclear Corp., Irvine, CA; (1-naphthyl-^{14}C)-carbaryl and (1-naphthyl-^{14}C)1-naphthol, Nuclear Chicago, Chicago, IL; (2-ring-^{14}C)diazinon, (2-ring-^{14}C)2-isopropyl-4-methyl-6-hydroxypyrimidine, (U-ring-^{14}C)atrazine, (U-ring-^{14}C)hydroxyatrazine, (U-ring-^{14}C)prometryn, (U-ring-^{14}C)ammeline and (U-ring-^{14}C)hydroxyprometryn, Ciba-Geigy Co., Greensboro, NC; (U-ring-^{14}C)isofenphos, Mobay Chemical Corp., Kansas City, MO; (7-^{14}C)salicylic acid and (U-ring-^{14}C)2,4,5-trichlorophenol, Sigma Chemical Co., St.Louis, MO; (U-ring-^{14}C) carbofuran, FMC Corp., Princeton, NJ. ^{14}C-carbofuran phenol was obtained by hydrolysis of ^{14}C-carbofuran in alcoholic potassium hydroxide, acidification, and extraction with ether (Metcalf et al., 1968). The structures and water solubilities of these compounds are presented in Tables I to III.

Table I. Structures of Chemicals Studied

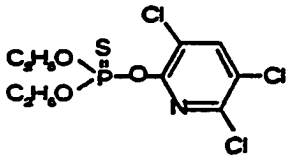
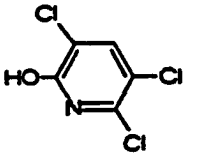
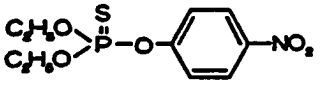
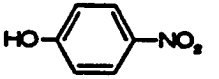
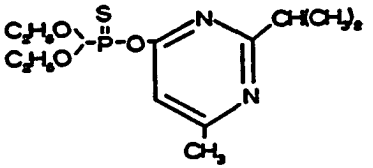
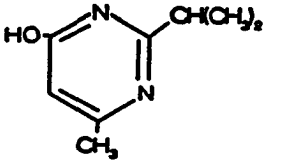
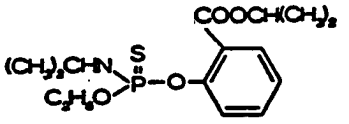
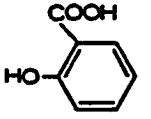
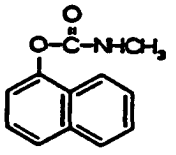
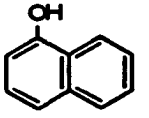
Pesticide	Chemical Structure	Hydrolysis Metabolite	Chemical Structure
chlorpyrifos		3,5,6-tricholoro-2-pyridinol	
parathion		p-nitrophenol	
diazinon		2-isopropyl-4-methyl-6-hydroxypyrimidine	
isofenphos		salicylic acid	
carbaryl		1-naphthol	

Table II. Structures of Chemicals Studied

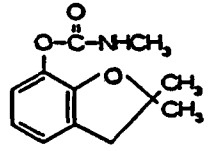
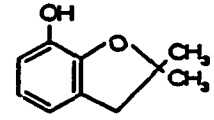
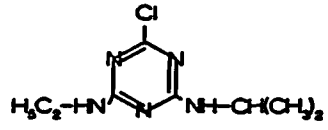
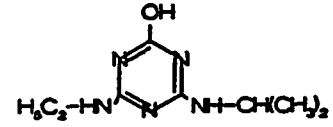
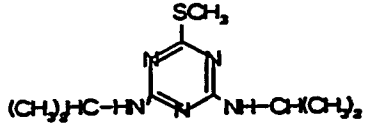
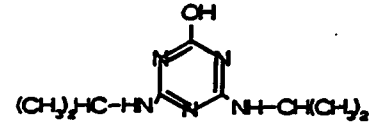
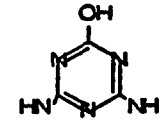
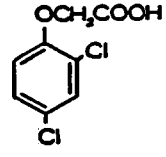
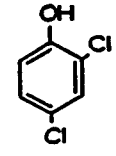
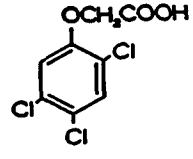
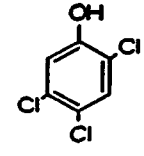
Pesticide	Chemical Structure	Hydrolysis Metabolite	Chemical Structure
carbofuran		carbofuran phenol	
atrazine		hydroxyatrazine	
prometryn		hydroxyprometryn	
		ammeline	
2,4-D		2,4-dichlorophenol	
2,4,5-T		2,4,5-trichlorophenol	

Table III. Water solubilities of chemicals studied

Pesticide	Solubility (ppm)	Hydrolysis Metabolite	Solubility (ppm)
chlorpyrifos	2[a,b]	3,5,6-tricholoro-2-pyridinol	220[a,c]
parathion	20[a,b]	p-nitrophenol	16,000[a,d]
diazinon	40[b,f]	2-isopropyl-4-methyl-6-hydroxypyrimidine	1,041[a]
isofenphos	24[b,f]	salicylic acid	2,174[a,b]
carbaryl	40[a,c]	1-naphthol	180[a]
carbofuran	700[a,b]	carbofuran phenol	3,871[a]
atrazine	70[a,b]	hydroxyatrazine	16[a,d]
prometryn	48[b,f]	hydroxyprometryn	15[a]
		ammeline	21[a,d]
2,4-D	890[a,g]	2,4-dichlorophenol	4,500[a,g]
2,4,5-T	238[b,e]	2,4,5-trichlorophenol	1,190[a,g]

[a]Water solubility at 25°C.

[b]Windholz et al. (1983).

[c]Kenaga and Goring (1980).

[d]Dr. Homer LeBaron, Ciba-Geigy, Greensboro, NC. (personal-communication).

[e]Water solubility at 30°C.

[f]Water solubility at 20°C.

[g]Verschueren (1983).

Soils

The six soils used in this study were selected to include a wide range in clay (8.0 to 34.1%) and organic matter (0.7 to 6.1%) contents. Five of these soils were collected from the A horizon and the sixth, a silt loam soil, was sampled from the C horizon. All the soils were air-dried, passed through a 1/4-inch-mesh sieve, and their soil properties were characterized (Table IV). Soil samples with small and homogeneous particle size were obtained by sieving the soils in a mechanical siever. Coarse-textured soils (loamy sand, sandy loam, and loam soils) were sieved thru 590 μm, and medium-and fine-textured soils (silt loam, silty clay loam, and clay loam soils) were sieved thru 250 μm. Helling (1971a) observed no change in mobility patterns due to the exclusion of medium and coarse sand fractions.

Soil thin-layer chromatography

Soil was slurried with water until moderately fluid and then applied directly to clean glass plates (20x20 cm). A uniform layer (500 μm thick) was obtained by moving a glass rod over masking tape along the plate edges. Plates were allowed to dry overnight at room temperature. Five to seven ^{14}C-labeled compounds (1-10 μg each containing 0.01 to 0.1 μCi) were applied as a spot with a capillary tube 3 cm from the bottom edge of each plate. Carbofuran was employed on each plate as an internal standard and four such repetitions were made for each soil type. A strip of paper towel, about 2 cm wide, was moistened with distilled water and wrapped around the bottom of the plates so that the lower 2 cm of soil was covered to prevent any sloughing. The plates were developed to 14 cm

Table IV. Properties of soils used in mobility studies

Soil type	sand %	silt %	clay %	OM %	pH	CEC meq/100g	% H_2O (1/3 bar)
Shelby clay loam	29.9	36.0	34.1	6.1	6.4	32.0	32.4
Readlyn loam	41.3	32.0	26.7	2.8	5.5	19.1	22.4
Zook silty clay loam	17.0	52.0	31.0	2.1	7.7	37.8	29.8
Dickinson sandy loam	72.0	18.0	10.0	2.0	5.5	8.6	15.2
Sparta loamy sand	83.0	9.0	8.0	1.2	8.3	4.8	10.4
Hamburg silt loam	5.0	82.0	13.0	0.7	8.5	14.9	22.8

with distilled water by ascending chromatography, and the developing time varied from 1 to 8 h depending on the soil type. The developed plates were air-dried and then exposed to Kodak Royal Blue X-ray film (8x10") for two to three weeks. The R_f value for each compound was measured as the front of the spot or streak in the resultant autoradiogram.

Water solubility studies

Water solubilities of carbofuran phenol, 1-naphthol, 2-isopropyl-4-methyl-6-hydroxypyrimidine, and hydroxyprometryn were estimated using the methods adapted from Bowman and Sans (1979) and Coats et al. (1979). Excess amounts of ^{14}C-labeled chemicals were placed in 500 ml glass bottles and the organic solvents were evaporated under dry nitrogen gas. The volume of distilled water used, per sample, varied from 100 to 250 ml, and the bottles were stirred on a magnetic stirrer. After 24 h the samples were centrifuged for 1 h at 25°C and counted in the liquid scintillation counter. There were four replicates for each chemical.

Statistical analysis

All the values for mobility and soil characteristics were subjected to a simple regression analysis to determine the ability of soil parameters to predict the mobility of chemicals in soil. R^2 values less than 50% do not contribute substantially in predicting the mobility; hence, the actual values were not included in the table. A multiple regression analysis was done to determine the predictive power of combinations of soil characteristics. For determining the best combination of predictors, importance was given for high R^2 values and for soil characteristics that contributed significantly to the prediction

model. To estimate the influence of water solubility on mobility, a simple regression analysis of R_f values and solubility data was done for all chemicals and for each group of chemicals.

RESULTS AND DISCUSSION

Comparative mobility of pesticies and their metabolites

The mobilities of the chemicals investigated in this study are expressed as R_f values in Tables V and VI. The R_f values ranged from 0.00 to 1.00, indicating the potential differences observed among the movement of the chemicals in soils. No prior mobility data were found for most of the insecticidal compounds and hydrolysis metabolites. Of the four organophosphorus insecticides studied, chlorpyrifos was immobile in all the soils studied and the other three insecticides (parathion, diazinon, and isofenphos) were slightly mobile with R_f values < 0.25. The hydrolysis products of these insecticides were significantly more mobile than their respective parent compounds ($p < 0.01$). In contrast, the metabolites of atrazine and 2,4-D were less mobile than their parent compounds. Hydroxyatrazine is more strongly retained by soil than atrazine (Armstrong et al., 1967), and this may be the reason for its low mobility. Helling (1971b) also found hydroxyatrazine to be less mobile than its precursor. 2,4-D was mobile in all six soils studied, whereas 2,4-dichlorophenol was low to intermediate in mobility. Both these compounds undergo rapid degradation, and there is no evidence of their accumulation in soil (Helling, 1971b; Somasundaram et al., 1989b).

Carbaryl and its hydrolysis metabolite, 1-naphthol, were not significantly different in mobility potential and exhibited low mobility in soils with higher clay contents (clay loam, loam, and silty clay loam). Both carbaryl and 1-naphthol form soil-bound residues, particularly in soils with a greater clay content (Murthy and Raghu, 1988). Carbofuran and carbofuran phenol were mobile in all the soils,

Table V. Mobility of pesticides and their hydrolysis metabolites in six soils

Pesticide/ metabolite	R_f value in					
	clay loam	loam	silty clay loam	sandy loam	loamy sand	silt loam
chlorpyrifos	0.02	0.02	0.02	0.02	0.02	0.00
t-pyridinol	0.26	0.26	0.58	0.36	0.94	1.00
parathion	0.10	0.10	0.14	0.12	0.18	0.13
p-nitrophenol	0.15	0.23	0.50	0.46	0.89	1.00
diazinon	0.10	0.12	0.12	0.17	0.24	0.21
h-pyrimidine	0.80	0.78	0.71	0.74	0.96	0.81
isofenphos	0.09	0.13	0.09	0.16	0.10	0.16
salicylic acid	0.09	0.39	0.59	0.35	0.75	0.96
carbaryl	0.11	0.15	0.17	0.24	0.62	0.53
1-naphthol	0.11	0.15	0.12	0.23	0.47	0.22

/ Variable does not offer significant explanation of R_f and $p > 0.05$.

+ $R^2 < 50$ % and $p < 0.05$.

sis metabolites in six soils, as determined by soil TLC technique

			R^2 (as %) for					
dy am	loamy sand	silt loam	clay (cl)	OM	pH	CEC	% H_2O (w)	Best combination (R^2)
)2	0.02	0.00	/	/	+	/	/	pH, CEC, w, OM (76)
36	0.94	1.00	+	51	84	/	+	pH, cl (97)
12	0.18	0.13	+	+	54	/	+	OM, CEC, pH, w (95)
36	0.89	1.00	54	65	69	+	+	cl, OM, pH (97)
.7	0.24	0.21	84	50	+	69	66	pH, CEC (98)
4	0.96	0.81	+	/	+	+	+	cl, OM, pH, CEC (90)
6	0.10	0.16	+	+	/	+	+	OM, pH, CEC, w (92)
5	0.75	0.96	+	+	84	/	/	pH, CEC, w (92)
4	0.62	0.53	+	+	59	+	+	pH, CEC (99)
3	0.47	0.22	65	+	+	64	77	OM, pH, w (97)

ion of R_f and $p > 0.05$.

Table VI. Mobility of pesticides and their hydrolysis metabolites in six soils, as

Pesticide/ metabolite	R_f value in						
	clay loam	loam	silty clay loam	sandy loam	loamy sand	silt loam	clay (cl)
carbofuran	0.57	0.76	0.69	0.77	0.81	0.75	66
carbofuran phenol	0.33	0.42	0.33	0.63	0.68	0.48	88
atrazine	0.21	0.28	0.44	0.44	0.61	0.64	60
hydroxyatrazine	0.00	0.00	0.02	0.02	0.02	0.18	/
Prometryn	0.11	0.03	0.34	0.13	0.60	0.49	+
hydroxyprometryn	0.00	0.00	0.10	0.02	0.00	0.23	/
ammeline	0.00	0.00	0.00	0.02	0.00	0.02	+
2,4-D	0.57	0.56	0.67	0.68	1.00	1.00	56
2,4-d-phenol	0.12	0.11	0.20	0.14	0.58	0.46	+
2,4,5-T	0.00	0.02	0.03	0.03	0.03	0.00	/
2,4,5-t-phenol	0.00	0.00	0.00	0.00	0.01	0.00	+

/ variable does not offer significant explanation of R_f and $p > 0.05$.

+ $R^2 < 50$ % and $p < 0.05$.

is metabolites in six soils, as determined by soil TLC technique

R^2 (as %) for							
loamy sand	silt loam	clay (cl)	OM	pH	CEC	% H_2O (w)	Best combination (R^2)
0.81	0.75	66	75	/	63	/	OM, w, cl (95)
0.68	0.48	88	+	/	89	76	OM, w, cl (98)
0.61	0.64	60	74	59	+	+	cl, OM, pH (97)
0.02	0.18	/	+	+	/	/	OM, pH, CEC, w (96)
0.60	0.49	+	+	88	/	/	pH, w (98)
0.00	0.23	/	+	+	/	+	OM, w, cl (93)
0.00	0.02	+	+	/	+	/	cl, pH, w, OM (93)
1.00	1.00	56	51	68	+	+	pH, CEC (97)
0.58	0.46	+	+	71	+	+	pH, CEC (96)
0.03	0.00	/	/	/	/	+	pH, CEC, w (89)
0.01	0.00	+	/	/	+	+	cl, pH, w (56)

n of R_f and $p > 0.05$.

and their mobility was significantly different only at p = 0.05. The mobility of prometryn was not statistically different from its metabolites (hydroxyprometryn and ammeline), although hydroxyprometryn's mobility was significant at p = 0.08. Weber et al. (1969) found no difference in the adsorption rates of prometryn and hydroxyprometryn to soil organic matter. 2,4,5-T and 2,4,5-trichlorophenol were not mobile in any of the soils studied.

Influence of soil charateristics on mobility

Soil properties such as organic matter, pH, clay, cation exchange capacity (CEC), and field moisture capacity have been widely reported to influence the behavior of pesticides in soil (Helling et al., 1971). The R_f values varied considerably among the different soil types studied, indicating the important role played by soil characteristics on mobility.

The best single predictor for each chemical varied among all five soil variables studied. Soil pH figured among the best combination of predictors for all but three chemicals and was the best single predictor for eight of the 21 chemicals studied. Most of the chemicals were more mobile in loamy sand and silt loam than in other soils. These two soils had relatively high pHs of 8.3 and 8.5, respectively. The pH has an indirect influence on movement by affecting stronger adsorption of chemicals at low pH values (Weber, 1970; Renner et al., 1988), resulting in lower mobility. Adsorption and mobility are interrelated, and mobility depends essentially on the degree of interaction between the pesticide molecules and soil components. In general, adsorption of

chemicals is weak at neutral pHs and above, leading to increased movement (Nicholls, 1988) and faster degradation in alkaline soils (Walker, 1987). Mobility of 2,4-D, picloram, dicamba, and fenac (Helling, 1971c) and simazine (Nearpass, 1965) in acidic to neutral soils were pH-related. In the mobility studies of Helling (1971c), soil pH seemed to be an important parameter only for the movement of organic acids. In the current studies, pH influenced the mobility of chemicals belonging to several different groups such as organophosphorus and carbamate insecticides, s-triazines, and phenoxyalkanoic acids.

In the simple regression analysis for determining the influence of organic matter on mobility, the organic matter coefficient (not shown in table) for all the chemicals studied was less than zero, indicating that greater organic matter content retarded mobility. Organic matter was among the best combinations of predictors for 12 of the 21 chemicals investigated. Organic matter has been cited by many researchers as one of the major factors in controlling the movement of pesticides in soil (Helling, 1971c; Jamet and Thoisy-Dur, 1988), and is also documented as the most active soil component in reducing the phytotoxicity of pesticides. Phytotoxicity occurs mainly through the availability of chemicals that are toxic to plants. Organic matter is implicated in reducing the availability by increasing adsorption (Hayes, 1970). However, greater organic matter contents need not always result in greater binding and low mobility in soil. Obrigawitch et al. (1982) compared the degradation of EPTC in soils with different organic matter contents and found the rate of degradation to be greater in soils with

more organic matter. Similarly, atrazine degradation rates also were found to be higher in soils of high organic matter (Armstrong et al., 1967). The higher degradation rate observed may be attributed to possible increases in (1) heterotrophic microorganisms, which increase in numbers with increased organic matter levels, and (2) cometabolic microorganisms, which require an additional energy source because they do not obtain energy from cometabolic degradation. Water soluble soil organic materials may act as vehicles for the transport of chemicals in soil (Madhun et al., 1986), thus enhancing the mobility/availability in soils rich in organic matter.

Mobility of most of the chemicals studied was low in clay loam, and this soil had a greater proportion of clay. The clay content was < 15% for sandy loam, loamy sand, and silt loam soils, and an increasing trend in the mobility of chemicals was observed in these soils. Clay coefficient for all chemicals tested was less than zero. Adsorption is often correlated with clay content, and mineral fractions of clay play an important role in adsorption. Organic matter has a stronger effect than clay on pesticide adsorption in soil. However, the adsorption of pesticides to clay is important, because most soils contain much more clay than organic matter, and clay contributes significantly to the total CEC (Helling, 1970). The influence of clay on pesticide adsorption and mobility is more evident in subsoils, which may be rich in clay (Nicholls, 1988).

Except for chlorpyrifos and hydroxyprometryn, cation exchange capacity coefficients were less than zero for all the chemicals studied.

CEC has been correlated negatively with movement of pesticides (Helling, 1971c) and positively with adsorption (Bailey and White, 1964); this relation among CEC, adsorption, and movement explains the effect of CEC on mobility.

Moisture content (at 1/3 bar) by itself was not a significant predictor of mobility for chlorpyrifos, salicylic acid, carbofuran, hydroxyatrazine, prometryn, and ammeline. However, it is included in the best combination of predictors for all these chemicals because, in combination with other variables, it contributes to more accurate predictions of mobility. Except for hydroxyprometryn, moisture content showed a negative coefficient for all chemicals. Similar negative correlations between moisture content and pesticide mobility were previously reported (Helling et al., 1971; Helling, 1971c). Savage (1976) found soil moisture content at 1/3 bar to be a better predictor of mobility and adsorption than sand, clay, organic matter, and pH. Because field moisture capacity is largely determined by the clay-organic matter complex, this relationship is expected.

For some chemicals such as the hydroxypyrimidine, hydroxyatrazine, and isofenphos, none of the soil parameters exceeded an $R^2 > 50\%$; however, combinations of more than two parameters did serve as better predictors.

Influence of pesticide characteristics on mobility

Pesticides similar in chemical structure may be expected to have the same mobility patterns. Helling (1971c) reported that the prediction of movement was often best for chemically similar pesticides. In this

study, hydroxyatrazine and hydroxyprometryn had nearly the same mobility pattern in all soils; similar observations were made by Helling (1971b). However, 2,4-D and 2,4,5-T, despite their structural similarity, differed in their potential for movement in soil. 2,4-D was very mobile (R_f=1.00) in loamy sand and silt loam and moderately mobile in all other soils. 2,4,5-T was immobile, irrespective of the soil types. Helling (1971b) found 2,4,5-T to be intermediate in mobility in silty clay loam soil (R_f=0.54). Although this value is much higher than the R_f value recorded for 2,4,5-T in the current studies, 2,4,5-T was consistently less mobile than 2,4-D and falls below the mobility category of other organic acids such as salicylic acid, picloram, and MCPA used in these studies. The addition of the third chlorine, at the number 5- position of the benzene ring in 2,4,5-T, may favor more adsorption, resulting in lower mobility. The increased mobility of 2,4-D may reflect lower adsorption, which renders it more readily available to microorganisms for further degradation, and it does not accumulate in soil (Somasundaram et al., 1989b). In general, acidic compounds are more mobile (Helling, 1971b), and the presence of the -COOH group in 2,4-D may be responsible for its increased mobility compared with 2,4-dichlorophenol.

Prometryn and atrazine are structurally similar s-triazines, but prometryn was less mobile than atrazine. Prometryn was reported to be bound to soil significantly more than many other triazines, including atrazine (Hayes, 1970), and this may have contributed to its low mobility. Weber (1970) observed that the amount of adsorption of triazines was dependent on the substituent in the 2- postion and was in

the order: $-SCH_3$ > $-OCH_3$ > -OH > -Cl. Adsorption studies with s-triazines revealed that the $-SCH_3$ group in prometryn influenced the electron density of the molecule to cause stronger binding forces between soil particles and the herbicide. Substitution of -Cl in the 2- position (propazine) resulted in increased movement (Harris, 1960), but substitution of -OH (hydroxyprometryn used in this study) did not increase the mobility. For chlorpyrifos, parathion, diazinon, and isofenphos, the presence of P=S in the alkyl moiety of these insecticides might have retarded the mobility, while their hydrolysis metabolites moved farther.

The influence of chemical structure on mobility is largely determined by the number, type, and position of functional groups. Presence of more than one aromatic ring, which may increase molecular size sufficiently to increase Van der Waals forces (Helling, 1971b), and amino and carbonyl groups, which may participate in hydrogen bonding and the introduction of double and triple bonds, all favor adsorption and lower mobility.

The water solubility of pesticides and other environmental contaminants has been generally suggested in the literature as one of the factors regulating the extent of mobility in soil. In this study a significant relationship ($p < 0.01$) existed between water solubility and mobility. However, solubility by itself may not be a good predictor of mobility as the R^2 was only 6%. The extent of influence of water solubility on mobility differed among the chemical groups in the order: s-triazines > organophosphosphates > carabamates > phenoxyalkanoic acids.

The hydrolysis metabolite of 2,4-D is about five times more soluble

than its parent compound, but it is less mobile than the parent compound (Table VI). Carbaryl and diazinon have the same water solubility (40 ppm), but carbaryl has a higher R_f value in loamy sand and silt loam soils. p-Nitrophenol (16,000 ppm) is nearly 25 times more soluble than carbofuran (700 ppm), yet p-nitrophenol moved much less in clay loam, loam, silty clay loam, and sandy loam soils. Similar observations illustrating the lack of correlation between solubility and pesticide movement have been reported (Harris, 1960; Rodgers, 1968). Direct correlation between solubility and movement of chemicals such as thiocarbamate herbicides and moderately polar solids with high melting points has been observed, but the correlation does not seem to be of general validity (Helling et al., 1971).

In a study investigating the movement and adsorption of herbicides in soil, Harris (1960) found adsorption to be a better predictor of herbicide mobility than their solubilities. Water solubility of a pesticide may not remain at the same level in soil, because it is affected by temperature, pH (Bowman and Sans, 1979), and water-soluble organic materials (Wershaw et al., 1969). In this study, atrazine and prometryn were less mobile as the soil pH decreased. Ward and Weber (1968) reported increased solubility of s-triazines with decreasing pH. This solubility - pH relationship supports our finding that, even if a pesticide is relatively more soluble than others, the solubility alone does not determine mobility. Nicholls (1988) is of the view that, despite being widely mentioned in the literature, water solubility is rarely an important predictor of pesticide mobility.

Implications of mobility on the availability and degradation of pesticides in soil

A compound's mobility is also an indicator of its potential for availability. Availability may influence a compound's susceptibility to microbial degradation. The hydrolysis metabolite and parent pesticide, whether toxic to the microorganisms or serving as a suitable substrate, should be available to the microorganisms in order to exert their toxicity or provide nutrient value.

Repeated application of p-nitrophenol (Sudhakar-Barik et al., 1979; Somasundaram et al., 1989b), 1-naphthol, and carbofuran phenol (Rajagopal et al., 1986), 2,4-dichlorophenol, and salicylic acid (Somasundaram et al., 1989b) has induced rapid degradation of their parent compounds. R_f values of these metabolites indicate intermediate to high mobility in soils. The p-nitrophenol, 2,4-dichlorophenol, and salicylic acid were utilized as energy sources by microorganisms and their availability in soils might have resulted in the induction of rapid microbial metabolism. Carbofuran phenol (Harris et al., 1984; Rajagopal et al., 1986; Somasundaram et al., 1989b) and 1-naphthol (Rajagopal et al., 1986) did not serve as microbial energy sources. Rajagopal et al. (1986) found these two metabolites also enhancing the degradation of their parent compounds. These two metabolites are freely available in soil, but the significance of their availability is yet to be understood.

The mobility data for isofenphos indicate that, for a compound to be susceptible to enhanced degradation, the compound need not necessarily be very mobile. Although isofenphos has a low mobility pattern, its

salicylic acid metabolite is more readily available to microorganisms. Salicylic acid is a benzoic acid analog, and its high availability is similar to that of benzoic acid and amiben (Bailey et al., 1968). Racke and Coats (1987) suggested that the formation of salicylic acid during isofenphos metabolism in soil may represent a key factor in the susceptiblity of isofenphos to enhanced degradation. The potential of salicylic acid to condition soils for rapid degradation of isofenphos has been observed in our laboratory (Somasundaram et al., 1989b). Some pseudomonads are reported to carry the salicylic acid degradative plasmid (Chakrabarty, 1972). Observations from the current mobility studies and the metabolism studies of Racke and Coats (1987) and Somasundaram et al. (1989b) indicate that the less mobile isofenphos may be hydrolytically metabolized by surface-soil microorganisms and that the resultant metabolites are more available to degrading microorganisms. The hydrolysis of isofenphos seems to be an important complicating factor influencing its field performance.

Hydroxypyrimidine, the hydrolysis product of diazinon, is more readily available in all soils tested and is mineralized by microbes (Sethunathan and Pathak, 1972). It is also relatively less toxic to microorganisms (Somasundaram et al., 1989a). Availability, low microbial toxicity, and susceptibility to microbial metabolism of this insecticidal metabolite may favor enhanced degradation of its parent compound in soils with populations of degrading microorganisms.

Chlorpyrifos is immobile in soil and is not susceptible to rapid degradation. Its hydrolysis product, trichloropyridinol, is more mobile

in all soils studied. Racke et al. (1988) observed mineralization of pyridinol to CO_2 and inhibition of this mineralization at higher doses. The nonpersistent nature of trichloropyridinol has been documented (Chapman and Harris, 1980b; Szeto et al., 1988). These findings on pyridinol activity in soil and R_f values indicate that pyridinol does not accumulate in soil.

s-Triazines have not been reported to undergo enhanced microbial degradation. Chemical hydrolysis of s-triazines is more common than microbial involvement. Atrazine and prometryn are intermediate in availability, and their resistance to enhanced degradation may owe to their microbial toxicity, low nutrient value, and/or metabolism to less-available hydrolytic products. The low availability of s-triazine hydroxy analogs may be responsible for their nonphytotoxic nature. Both 2,4,5-T and its phenolic metabolite are not freely available and may remain in soil for a longer time, indicating their unavailability for microbial degradation.

In summary, mobility data of these chemicals based on soil thin-layer chromatography can be of value in predicting the chemical's movement through a soil and their persistence in that soil. The compounds that are more mobile on STLC are bound less tightly, and consequently are more available for microbial degradation. The pesticides and metabolites that are less mobile are less available for microbial degradation, but more susceptible to chemical degradation by the mineral components of the soil. Information derived from the comparative studies on the pesticides and their metabolites in various soil types can be of predictive value

regarding persistence and degradation, as well as the potential for leaching.

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CHAPTER III. APPLICATION OF THE MICROTOX SYSTEM TO ASSESS THE TOXICITY OF PESTICIDES AND THEIR HYDROLYSIS METABOLITES

ABSTRACT

The Microtox system was employed to determine the toxicity of 21 chemicals (seven insecticides, two herbicides, and 12 of their hydrolysis metabolites) to *Photobacterium phosphoreum*. Some of the metabolites studied (3,5,6-trichloro-2-pyridinol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, and isopropyl salicylate) were more toxic to *P. phosphoreum* than their respective parent compounds. The hydrolysis metabolites salicylic acid, 2-isopropyl-4-methyl-6-hydroxypyrimidine, carbofuran phenol, and methylamine were less toxic to *P. phosphoreum*. The toxicity of s-triazine metabolites could not be determined because of their low solubility. The range in toxicity among the metabolites reflects their potential in affecting the microbial degradation of their parent compounds. The Microtox system is not suitable for assessing the toxicity of less water-soluble compounds.

INTRODUCTION

The soil microorganisms, bacteria in particular, play an important role in the environmental fate of soil-applied pesticides. The ability of bacteria to metabolize pesticides and/or their metabolites for their benefit has been well documented (Sudhakar-Barik et al., 1979; Racke and Coats, 1987). One result of this catabolism is the failure of some pesticides to adequately control the target pests because of decreased persistence (Felsot, 1989). One of the properties of pesticides and their metabolites that may influence the induction or inhibition of enhanced microbial degradation is their toxicity to the soil microbes that are responsible for degradation.

Inasmuch as it would be expensive and time consuming to determine the toxicities of pesticides and their metabolities to all possible soil bacteria involved in the enhanced degradation process, we used the Beckman Microtox system to assess their relative toxicities to a model bacterium. This system utilizes *Photobacterium phosphoreum*, a marine bacterium that is phylogenetically related to several genera of bacteria important in soil. The Microtox system was originally developed to assess the toxic effects of complex industrial effluents (Bulich, 1984). Since its introduction in 1979, the application of the Microtox system has been extended to determine the toxicity of aquatic pollutants, waste waters, fossil-fuel process waters, mycotoxins, and numerous other chemicals (Ribo and Kaiser, 1987).

In this study, we used the Microtox analyzer to determine the relative microbial toxicities of some pesticides and their metabolites.

Pesticides known to be susceptible to enhanced degradation and others that have displayed apparent resistence to enhanced degradation were included in this study. Because hydrolysis is a significant step in the chemical and microbial degradation of pesticides in soil (Matsumura, 1980), the principal focus of this investigation was on hydrolytic metabolites.

MATERIALS AND METHODS

Twenty-one chemicals (seven insecticides, two herbicides, and 12 of their hydrolysis metabolites) were used in this study. The chemicals obtained from their respective sources were: 1-naphthol, 4-nitrophenol, thiophenol, 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4-dichlorophenol, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,4,5-trichlorophenol, Aldrich Chemical Company Inc., Milwaukee, WI; carbaryl, fonofos, and parathion, U.S. Environmental Protection Agency, Research Triangle Park, NC; methyl phenyl sulfone, Stauffer Chemical Co., Mountain View, CA; carbofuran and carbofuran phenol, FMC Corp., Princeton, NJ; methylamine, and sodium salicylate, Fisher Scientific Co., Itasca, IL; chlorpyrifos and 3,5,6-trichloro-2-pyridinol, Dow Chemical Co., Midland, MI; isofenphos and isopropyl salicylate, Mobay Chemical Corp., Kansas City, MO; diazinon and 2-isopropyl-4-methyl-6-hydroxypyrimidine, Ciba-Geigy Co., Greensboro, NC.

The Microtox test was performed using the Beckman Model 2055 Microtox Toxicity Analyzer and by following the procedures detailed in Beckman's operating manual (Anonymous, 1979). The freeze-dried bacteria, reconstitution solution (organic-free distilled water), and diluent (a solution containing 20 g L^{-1} NaCl to provide osmotic stability for the marine bacterium) were purchased from Microbics Corporation, Carlsbad, CA.

The Microtox system measures the light emitted from bioluminescent *Photobacterium phosphoreum* that have been exposed to a chemical dissolved in the diluent. The details of theory and operation of the Microtox

analyzer and of reagents used have been described (Anonymous, 1979; Bulich et al., 1981). The incubator block and the chamber in which light production is measured were maintained at a temperature of 15 ± 0.1°C. The toxicity end-point is the 5-min median effective concentration (EC_{50}), which is the concentration that causes a 50% reduction in light output. The toxicities were determined according to the procedure described by Bulich et al. (1981).

The chemicals were dissolved in diluent, and a dilution series consisting of five concentrations was prepared for each chemical. Each of these dilution sets included one concentration that caused a 40 to 60% light reduction. Less water-soluble compounds were first dissolved in methanol or DMSO and then serially diluted with the diluent. The concentration of the organic solvents did not exceed 8% (vol/vol) in the test samples. Our preliminary investigations showed that concentrations of solvent vehicles exceeding 8% were toxic to the bacteria. Each test was carried out twice for all chemicals, and in some cases three to four times until the EC_{50} values differed by not more than 20% from each other.

RESULTS AND DISCUSSION

The toxicities of the pesticides and their metabolites investigated in this study are expressed as EC_{50} (μg/ml) values in Table I. The available information on the microbial toxicity of pesticides and their metabolites is limited, and no prior data were found for 12 of the 21 chemicals studied.

Despite their structural similarity, 2,4-D and 2,4,5-T differed greatly in their microbial toxicity. Higher EC_{50} values indicate low toxicity, and the EC_{50} values for 2,4-D and its hydrolysis metabolite were 2- and 2.5-fold higher than those of 2,4,5-T and its hydrolysis metabolite. Similar observations of increased toxicity of 2,4,5-T and its phenolic metabolite have been reported (Ribo and Kaiser, 1983). Ruckdeschel et al. (1987) found 2,4,5-trichlorophenol 2- to 12-fold more toxic than 2,4-dichlorophenol to 28 of 30 bacterial strains studied.

The increased toxicity of 2,4,5-T and its phenolic metabolite can be attributed to the presence of a third chlorine at the number 5- position of the benzene ring. Similar observations relating toxicity to the number and position of chlorine substitutions have been reported for other chlorophenols (Milner and Goulder, 1986), and chlorobenzenes (Ribo and Kaiser, 1983).

Except for chlorpyrifos and isofenphos among the seven insecticides studied, all were relatively toxic to *P. phosphoreum* (EC_{50} < 21 ppm). Because most of the pesticides are degraded into breakdown products in soil and because some of these products are more persistent than the parent compounds (Khan and Saidak, 1981; Somasundaram et al., 1987), the microbial toxicity of metabolites is of practical significance.

Table I. The toxicity of pesticides and their metabolites as determined with the Microtox system

Pesticide	EC_{50}	Hydrolysis metabolite	EC_{50}
2,4-D	101	2,4-dichlorophenol	5
2,4,5-T	52	2,4,5-trichlorophenol	2
carbaryl	5	1-naphthol	4
fonofos	5	methyl phenyl sulfone	3
		thiophenol	5
parathion	9	4-nitrophenol	14
chlorpyrifos	46	3,5,6-trichloro-2-pyridinol	19
carbofuran	21	carbofuran phenol	61
		methylamine	35
isofenphos	98	salicylic acid	214
		isopropyl salicylate	6
diazinon	10	2-isopropyl-4-methyl-6-pyrimidine	886

Some of the metabolites studied (3,5,6-trichloro-2-pyridinol, 2,4-dichlorophenol, 2,4,5-trichlorophenol, and isopropyl salicylate) were more toxic to *P. phosphoreum* than their respective parent compounds. The EC_{50} values of these metabolites ranged from 2 to 19 ppm. The toxicity of 1-naphthol to *Pseudomonas* sp., *Nitrosomonas* sp., and *Nitrobacter* sp. has been reported (Ramakrishna and Sethunathan, 1983). 1-Naphthol does not seem to serve as an energy source for microorganisms. This is evident from its accumulation in rice soils after repeated applications (Rajagopal et al., 1986). Repeated applications of 3,5,6-trichloro-2-pyridinol to soil resulted in less mineralization and increased persistence of chlorpyrifos (Somasundaram et al., 1989). This may be because of the toxicity of the pyridinol metabolite to soil microorganisms. 2,4-Dichlorophenol was 20- fold more toxic than its parent compound to *P. phosphoreum*. Tyler and Finn (1974) found that 2,4-dichlorophenol inhibited the growth of *Pseudomonas* sp. at 25 ppm, whereas 2,4-D had no effect on growth at concentrations up to 2000 ppm. Although 2,4-dichlorophenol was more toxic in these studies, recent investigations in our laboratory have indicated the nutritive value of 2,4-dichlorophenol and its ability to induce rapid degradation of 2,4-D (Somasundaram et al., 1989). Similarly, 4-nitrophenol that has a low EC_{50} value (14 ppm) was utilized as an energy source by microorganisms, and its application resulted in an increase in the population of parathion-hydrolyzing microbes (Sudhakar-Barik et al., 1979).

Salicylic acid and 2-isopropyl-4-methyl-6-hydroxypyrimidine yielded higher EC_{50} values, reflecting their low toxicity to bacteria. Some soil

bacteria carry degradative plasmids for salicylic acid metabolism (Chakrabarty, 1972), and repeated applications of salicylic acid induced the enhanced degradation of its parent organophosphorus compound, isofenphos (Somasundaram et al., 1989). The highest EC_{50} value among all the chemicals studied was observed for the hydrolysis metabolite of diazinon (2-isopropyl-4-methyl-6-hydroxypyrimidine). The ability of microorganisms to mineralize this metabolite to CO_2 has been demonstrated previously (Sethunathan and Pathak, 1972).

The Microtox test seems to be quicker than other methods in estimating the toxicity of chemical compounds to bacteria. The Microtox test was also more sensitive compared with other bacterial bioassays (Dutka and Kwan, 1981; Ribo and Kaiser, 1983). For example in Dutka and Kwan's study, the EC_{50} value for 1-naphthol was 3.80 ppm in the Microtox test as compared with > 100 ppm in a *Pseudomonas fluorescens* bioassay. Because of this increased sensitivity, some researchers are of the opinion that Microtox data should be restricted to rank chemicals according to their comparative toxicity and not for predictive purposes (King, 1984).

An important limitation of Microtox system is that only the toxicity of aqueous solutions can be determined. Addition of organic solvents to increase the solubility of chemicals resulted in increased toxicity to *P. phosphoreum*. In this study, we could not determine the EC_{50} values for s-triazine metabolites (hydroxyprometryn and hydroxyatrazine) inasmuch as these compounds did not dissolve in the diluent, 8% methanol, or 8% DMSO (the solvents that do not interfere with toxicity determination). s-

Triazine metabolites dissolve only in acidic solutions or other organic solvents such as chloroform (which are toxic to *P. phosphoreum*). Saturated solutions of these s-triazine metabolites in the diluent or methanol or DMSO did not produce any measurable effect. The difficulty in determining the toxicity of less-soluble compounds in Microtox tests has been observed by many other investigators (Qureshi et al., 1984; Ribo and Kaiser, 1987).

In summary, the toxicity data generated in this study provide an indication of the potential toxicity of pesticide metabolites to bacteria. The EC_{50} values for 3,5,6-trichloro-2-pyridinol and salicylic acid and the metabolism studies of these chemicals conducted in our laboratory indicate that metabolites may play an important role in the induction or inhibition of enhanced microbial degradation of some pesticides. Some hydrolysis products that have been reported to serve as energy or nutrient sources for soil microbes displayed low EC_{50} values in this study. The toxicity of metabolites to *P. phosphoreum* may not correspond to toxicity to soil bacteria in all cases. The Microtox system is not suitable for assessing the toxicity of less-water-soluble compounds, and in general, the data should be interpreted with caution because of the system's high sensitivity and differences in susceptibility to chemicals among the different genera of bacteria.

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CHAPTER IV. EFFECT OF MANURING ON THE PERSISTENCE AND DEGRADATION OF SOIL INSECTICIDES

ABSTRACT

Laboratory experiments were conducted to investigate the short- and long-term effects of hog manuring on the persistence and degradation of seven soil insecticides. Continued history of manuring for seven years resulted in increased persistence of carbofuran, chlorpyrifos, fonofos, terbufos, ethoprop, and phorate or their insecticidal metabolites. The degradation of isofenphos was not greatly influenced by manuring. The results showed no evidence that amending soil with manure encourages the development of enhanced microbial degradation of pesticides. The greater persistence of insecticides in manure-history soil may be due to its higher organic matter content and lower pH, and less selection pressure for pesticides inasmuch as nutrients may forestall development of adapted species of microbes.

INTRODUCTION

The use of soil-applied insecticides is a widely adopted management strategy to control insect pests in corn. A recent problem that has developed with continued use of an insecticide is the phenomenon of enhanced degradation by soil microorganisms (Felsot et al., 1981). Enhanced degradation occurs when a soil-applied pesticide is more rapidly degraded by soil microorganisms that have previously been exposed to the same pesticide or class of pesticides. Several factors such as microorganisms present, soil texture, moisture, and pH govern the enhanced degradation of soil insecticides. One factor of unknown importance is the application of manures to agricultural soils.

Manuring is a commonly adopted agronomic practice in crop production. It influences a number of factors, including microbial activity, microbial diversity, and physical, chemical, and biological characteristics of soil. Because soil characteristics such as organic matter content and pH affect the rate of pesticide degradation (Anderson, 1981; Stevenson, 1972), manure application may alter the rate and (or) mechanism of pesticide degradation. Previous research on the effect of manures on pesticide degradation has been mainly from the waste-disposal point of view, focusing on the fate of persistent pesticides as affected by laboratory amendments at extremely high loading rates (Doyle et al., 1978, 1981). This study was initiated to determine the short- and long-term effects of recommended field loading rates of hog manure on the persistence and degradation of seven soil insecticides in comparison with nonmanured soil.

MATERIALS AND METHODS

Treatments

Four treatments were considered. The first two used field-collected soil with and without manuring history. The second two used soil with no manuring history but to which two loading rates of fresh dried manure were added in the laboratory. Two field-collected Tama silt loam soils, one with seven continuous years of hog manuring (pH 5.8; organic matter 5.6%) and the second with no history of manuring for the seven year period (pH 7.3; organic matter 4.2%), were collected at the begining of the 1986 growing season from the same field near Stanwood, Iowa. The soil with a history of manuring received hog manure equivalent to 180 lb of nitrogen/acre/year. The soils were sieved to remove debris, and stored at 4^0C until used.

For the fresh-manure additions, hog manure was collected from the Swine Nutrition farm at Iowa State University, Ames, IA. The manure was oven-dried at 65^0C to facilitate uniform mixing with the soil. The quantity of manure used in the laboratory amendments was based on the nitrogen requirements of the corn crop (N=207 kg/Ha) and the nitrogen content of the dry hog manure (N=3.1%). This gave a recommended loading rate of 6,700 kilograms of dry hog manure per hectare (1x); a rate 10 times higher (10x) was also included. The manure was uniformly mixed with the field-collected soil, which had no history of manuring and incubated aerobically at 25^0C for a two-week period. Additionally, soils with a history and no history of manuring were also incubated under similar conditions.

Chemicals

After a two-week incubation, a 50-g dry-weight portion of each soil was separately treated with each of the seven ^{14}C-insecticides in acetone at 5 ppm as described by Lichtenstein and Schultz (1959). The following radiolabelled insecticides were obtained from the respective manufacturers: ^{14}C-U ring-carbofuran (FMC Corporation, Princeton, NJ), ^{14}C-2,6-phenyl-chlorpyrifos (Dow Chemical Company, Midland, MI), ^{14}C-ethyl-1-ethoprop (Rhone-Poulenc Inc., Monmouth Junction, NJ), ^{14}C-U ring-fonofos (Stauffer Chemical Company, Mountain View, CA), ^{14}C-methylene-terbufos and ^{14}C-methylene-phorate (American Cynamid Corporation, Princeton, NJ), and ^{14}C-U ring-isofenphos (Mobay Chemical Company, Kansas City, MO). The radiopurity of each chemical was greater than 98%.

Soil incubation and analyses

After insecticidal additions, aliquots of treated soils were taken for incubation and also for analysis of initial insecticide concentration. Each treatment was replicated four times, and the treated soils were placed in 8-oz French square bottles. Soils were moistened to field capacity (1/3 bar soil moisture tension) with distilled water, and glass vials containing 0.1N NaOH were placed in each bottle to trap the CO_2 evolved. The bottles were closed tightly and incubated at 25°C for three weeks. The CO_2 traps were replaced daily for the first week and on alternate days for the subsequent two weeks and then analyzed for $^{14}CO_2$ by liquid scintillation counting.

At the end of the incubation period, ^{14}C-insecticide residues in the soil were extracted twice with acetone-methanol (1:1) and once with

acetone-methanol-dichloromethane (1:1:1) and partitioned into dichloromethane as described by Lichtenstein et al. (1973). Thin-layer chromotography and autoradiography techniques, as described by Hsin and Coats (1986), were used to characterize carbofuran (Koeppe and Lichtenstein, 1984), chlorpyrifos, ethoprop, fonofos, phorate, terbufos, isofenphos (Racke and Coats, 1988), and their respective metabolites.

Microbial enumeration

The influence of manuring on the total microbial numbers was determined by using soil dilution plate counts. Nutrient agar was used for culturing soil bacteria, and rose bengal agar was used for culturing soil fungi. Final plate counts were taken after one week, and each dilution was replicated thrice.

RESULTS AND DISCUSSION

Virtually no carbofuran was recovered in the soils with no manure-history and laboratory amendments (Table I). However, 21% of the applied ^{14}C-carbofuran persisted in manure-history soil. The field in which the soils were collected had had a carbofuran failure (rootworm control had been ineffective), and the increased persistence in the manure-history soil suggested that manuring inhibited the development or expression of enhanced degradation. In all the treatments, a large portion of the applied ^{14}C-carbofuran was converted to soil-bound residues. Although the bound residues are subject to further degradation and release by soil microorganisms (Racke and Lichtenstein, 1985), their insecticidal activity is drastically reduced (Lichtenstein et al., 1977). Less binding was observed in manure-history soil, and, in general, the proportion of soil-bound residues formed was inversely related to the insecticide degradation in soil.

A two-fold increase in the persistence of chlorpyrifos was observed in soil with manure history as compared with nonmanured soil (Table II). The hydrolysis product of chlorpyrifos (3,5,6-trichloropyridinol) was present at a concentration four times higher in nonmanured soil than in soil with a history of manuring. The persistence of the parent compound in laboratory amendments was greater than that in nonmanured soil but significantly less than that in soil with a manure history. Relative binding of ^{14}C-chlorpyrifos was relatively less in manure-history soil.

Table I. Effect of manuring on the persistence and degradation of [^{14}C]carbofuran during a three-week incubation

Fraction	^{14}C recovered, % of applied [^{14}C]carbofuran				
	No manure	1x	10x	7 years	LSD[1]
Soil					
Carbofuran	0.25[a]	0.71[a]	0.18[a]	21.44[b]	0.75
3-ketocarbofuran phenol	1.09[a]	0.15[b]	0.91[c]	2.95[d]	0.17
Soil-bound	74.07[a]	75.20[a]	78.94[b]	60.76[c]	3.67
$^{14}CO_2$	13.85[a]	12.11[b]	11.74[bc]	11.21[c]	0.62
Others[2]	1.82[a]	1.22[b]	1.59[ab]	2.65[c]	0.45
Total	91.08[a]	89.39[a]	93.36[b]	99.01[c]	2.06

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[1]LSD: Least significant difference.

[2]Includes volatile products other than $^{14}CO_2$ as well as polar, water soluble products.

Table II. Effect of manuring on the persistence and degradation of [^{14}C]chlorpyrifos during a three-week incubation

Fraction	^{14}C recovered, % of applied[^{14}C]chlorpyrifos				
	No manure	1x	10x	7 years	LSD[1]
Soil					
Chlorpyrifos	34.66[a]	36.63[a]	47.93[b]	70.83[c]	5.21
3,5,6-t Pyridinol	28.95[a]	23.06[b]	14.15[c]	7.07[d]	4.04
Soil-bound	17.12[a]	17.73[a]	17.16[a]	7.23[b]	0.63
$^{14}CO_2$	12.64[a]	11.75[b]	7.25[c]	10.26[d]	0.47
Others[e]	6.06[a]	9.23[b]	10.98[b]	3.31[c]	2.60
Total	99.43	98.40	97.47	98.70	NS

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-keuls Test).

[1]LSD: Least significant difference.

[2]Includes volatile products other than $^{14}CO_2$ as well as polar, water soluble products.

[3]NS: Not significant.

Fonofos was more persistent in soil with a history of manuring. Laboratory amendments resulted in lesser persistence of the parent fonofos as compared with nonmanured soil (Table III). The increased fonofos degradation in these treatments resulted in a greater production of $^{14}CO_2$ and soil-bound residues. The short-term effect of manuring on fonofos degradation observed in the current investigation was similar to the results of Lichtenstein et al. (1982).

At the end of the three-week incubation, nearly all the terbufos applied was metabolized to its primary and secondary oxidative products, terbufos sulfoxide and terbufos sulfone (Table IV). Soil with a history of manuring had increased levels of sulfoxide and decreased levels of sulfone as compared with the other treatments. Addition of manure at the rate of 67,000 kilograms per hectare resulted in increased levels of sulfone and decreased levels of sulfoxide. In soil with manure history, 72% of the applied ^{14}C-terbufos was recovered as sulfoxide and sulfone, and a lower concentration of bound residues was recovered.

Sixty-three percent of the applied ^{14}C-ethoprop was persistent in manure-history soil, which was 2.5- to 4.2-fold higher than in the other three treatments (Table V). The increased persistence was also indicated by low levels of $^{14}CO_2$ and bound residues. Ethoprop degradation was rapid in nonmanured and laboratory-amended soils, with about 50% of the applied insecticide being mineralized to $^{14}CO_2$.

Table III. Effect of manuring on the persistence and degradation of [^{14}C]fonofos during a three-week incubation

Fraction	^{14}C recovered, % of applied [^{14}C]fonofos				
	No manure	1x	10x	7 years	LSD[1]
Soil					
Fonofos	46.15[a]	37.33[b]	28.74[c]	52.76[d]	4.56
Methyl phenyl sulfone	4.53[a]	5.68[ab]	7.48[bc]	9.78[c]	2.84
Soil-bound	23.88[a]	25.43[b]	30.67[c]	18.21[d]	0.99
$^{14}CO_2$	19.44[a]	19.86[a]	23.27[b]	10.44[c]	0.56
Others[2]	1.58[a]	1.57[a]	2.35[ab]	3.00[ab]	1.30
Total	95.58[a]	89.87[b]	92.51[ab]	94.19[a]	3.72

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[1]LSD: Least significant difference.

[2]Includes volatile products other than $^{14}CO_2$ as well as polar, water soluble products.

Table IV. Effect of manuring on the persistence and degradation of [^{14}C]terbufos during a three-week incubation

Fraction	^{14}C recovered, % of applied [^{14}C]terbufos				
	No manure	1x	10x	7 years	LSD[1]
Soil					
Terbufos	0.58[a]	0.45[a]	0.99[b]	0.45[a]	0.19
Terbufos sulfoxide	45.99[a]	42.52[b]	20.02[c]	56.20[d]	2.62
Terbufos sulfone	18.22[a]	21.04[b]	32.54[c]	14.71[d]	1.95
Soil-bound	13.24[a]	15.96[b]	23.42[c]	9.93[d]	0.91
$^{14}CO_2$	12.04[a]	13.35[b]	12.78[ab]	13.24[b]	1.01
Others[2]	5.02[a]	5.75[b]	7.07[c]	4.07[d]	0.59
Total	95.09[a]	99.07[b]	96.82[ab]	98.60[b]	2.45

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[1]LSD: Least significant difference.

[2]Includes volatile products other than $^{14}CO_2$ as well as polar, water soluble products.

Table V. Effect of manuring on the persistence and degradation of [^{14}C]ethoprop during a three-week incubation

Fraction	^{14}C recovered, % of applied [^{14}C]ethoprop				
	No manure	1x	10x	7 years	LSD[1]
Soil					
Ethoprop	25.15[a]	20.39[b]	15.07[c]	62.78[d]	4.12
Soil-bound	26.93[a]	27.94[a]	27.12[a]	16.02[b]	1.41
$^{14}CO_2$	47.76[a]	51.97[b]	50.57[ab]	20.83[c]	3.22
Total	99.84[a]	100.30[a]	92.76[b]	99.63[a]	2.03

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[1]LSD: Least significant difference.

Phorate was rapidly oxidized to its metabolites, phorate sulfoxide and phorate sulfone (Table VI). The level of sulfone was greater than that of sulfoxide in all the treatments. As in terbufos, soil with a history of manuring had greater persistence of the metabolites resulting in significantly less recovery of $^{14}CO_2$ and bound residues. The relatively shorter persistence of the parent terbufos and phorate in soil and the role played by their oxidative metabolite towards target-pest activity has been previously reported (Harris and Chapman, 1980).

The degradation of isofenphos was not greatly influenced by manuring (Table VII). Although enhanced microbial degradation of isofenphos because of repeated use has been reported (Chapman et al., 1986; Racke and Coats, 1987), isofenphos is highly persistent in the first year of application. More than 65% of the applied parent insecticide persisted in all the treatments at the end of the three-week incubation. Significantly lesser amounts of the bound residues and $^{14}CO_2$ were recovered in soil with a manure history.

Application of manure for seven continuous years in the field did not significantly increase the total number of bacteria and fungi as compared with nonmanured soil (Table VIII). Bacteria have been reported to be the most important group of microorganisms in enhanced biodegradation of pesticides (Racke and Coats, 1987). Manure may serve as a readily available energy source for the existing bacterial population, reducing the probability of a selective advantage developing for bacteria that can ultilize a pesticide as a nutrient source.

Table VI. Effect of manuring on the persistence and degradation of [^{14}C]phorate during a three-week incubation

Fraction	^{14}C recovered, % of applied [^{14}C]phorate				
	No manure	1x	10x	7 years	LSD[1]
Soil					
Phorate	0.07[a]	0.14[ab]	0.10[a]	0.18[b]	0.07
Phorate sulfoxide	5.43[a]	7.05[a]	7.94[a]	16.02[b]	4.49
Phorate sulfone	45.50[a]	43.27[a]	33.10[b]	54.71[c]	5.27
Soil-bound	18.69[a]	18.89[a]	28.76[b]	11.64[c]	1.17
$^{14}CO_2$	19.70[a]	20.85[b]	21.37[b]	14.90[c]	0.83
Others[2]	8.16[ab]	8.88[a]	7.64[b]	2.53[c]	0.91
Total	97.55[a]	99.06[ab]	98.91[ab]	99.98[b]	1.62

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[1]LSD: Least significant difference.

[2]Includes volatile products other than $^{14}CO_2$ as well as polar, water soluble products.

Table VII. Effect of manuring on the persistence and degradation of [^{14}C]isofenphos during a 3-week incubation

Fraction	^{14}C recovered, % of applied [^{14}C]isofenphos				
	No manure	1x	10x	7 years	LSD[1]
Soil					
Isofenphos	70.43[a]	73.72[b]	66.92[c]	70.31[a]	3.25
Isofenphos oxon	7.97[a]	9.62[b]	6.49[c]	10.19[b]	1.02
Soil-bound	6.79[a]	7.73[b]	9.33[c]	4.94[d]	0.39
$^{14}CO_2$	4.46[a]	6.20[b]	7.53[c]	3.96[d]	0.12
Others[2]	0.39	0.40	0.22	0.24	NS[3]
Total	90.04[a]	97.67[b]	90.49[a]	89.64[a]	3.37

[a-d]Means in each row with the same letter are not significantly different at 5% level (Student-Newman-Keuls Test).

[1]LSD: Least significant difference.

[2]Includes volatile products other than $^{14}CO_2$ as well as polar, water soluble products.

[3]NS: Not significant.

Table VIII. Influence of manuring on total microbial numbers

Treatment	Number per gram dry weight soil	
	Bacteria (10^6)	Fungi (10^4)
No manure	6.6[a]	5.0[a]
1x	101.0[b]	32.0[b]
10x	291.0[c]	47.0[c]
7 Years	10.0[a]	8.3[a]
LSD[1]	15.8	5.4

[a-c]Means in each column with the same letter are not significantly different at the 5% level (Student-Newman-Keuls Test).

[1]LSD: Least significant difference.

Laboratory manure treatments of a soil resulted in 15- and 44-fold increases in bacterial numbers at 1x and 10x loading rates per hectare, respectively, as compared with nonmanured soil. The fungal population also increased by 6- and 9-fold at the two loading rates over that of nonmanured soil. This stimulation of microbial activity in laboratory-amended soils may be responsible for the increased breakdown of fonofos, terbufos, and phorate in these soils. Increased degradation of permethrin (Doyle et al., 1981) and carbofuran (Koeppe and Lichtenstein, 1984) in dairy manure-amended soils has been reported. However, these researchers used extremely high loading rates (15-fold higher than the recommended rate) and focused mainly on the short-term effects of manuring on pesticide degradation.

Organic matter is a major adsorbent in the soil, and persistence of soil insecticides may be positively correlated with the organic matter content. Soil that received manure for seven years had a higher organic matter content of 5.6% as compared with 4.2% in nonmanured soil. This increase in organic matter content may be an additional reason for the increased persistence in manure-history soils.

Continued history of manuring lowered the soil pH from 7.3 in non-manured soil to 5.8 in soil with manure history. The pH of laboratory-amended soil was not significantly lowered, and it remained at 6.9 and 7.2 for the two loading rates used. Soil pH may directly or indirectly influence the degradation of insecticides by affecting the ionic or molecular character of the chemical, the cation exchange capacity, or the ability of the microbial population to respond to a given chemical.

Getzin (1973) reported that carbofuran broke down much faster in alkaline than in acid soil and also observed that microbial action was involved. The increased rate of carbofuran degradation was inhibited by lowering the pH to below 6.0 (Read, 1983). Walker et al. (1986) found slow degradation of two fungicides (iprodione and vinclozolin) in soils with a pH below 5.5. Strong adsorptive bonds between the insecticides and soil colloids, coupled with low activity of degrading bacterial populations, may contribute to the increased persistence in acidic soils. Isofenphos, a phosphoramidate, is more acid-labile than other insecticides studied, yet also persisted in the lower-pH soil.

In summary, the three possible explanations for greater persistence of the organophosphorus and carbamate insecticides in a soil with a seven year history of manuring include (1) less selection pressure for pesticides inasmuch as nutrients may forestall development of adapted species of microbes, (2) higher organic matter may contribute to greater binding of the insecticides making them less available for microbial degradation, (3) lower pH may reduce rates of chemical and biological degradation. It is possible that all three factors contributed to the increased persistence. This study found no evidence that amending soil with manure encourages the development of enhanced microbial degradation of pesticides.

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SUMMARY

In an experiment designed to evaluate the potential of hydrolysis metabolites on conditioning the soil for enhanced microbial degradation, 2,4-dichlorophenol, p-nitrophenol, and salicylic acid conditioned the soil for enhanced degradation of their respective parent compounds. Pretreatment of soil with 3,5,6-trichloro-2-pyridinol resulted in increased persistence of its parent compound, chlorpyrifos. Prior exposure of soil to carbofuran phenol, 2-isopropyl-4-methyl-6-hydroxypyrimidine, methyl phenyl sulfone, thiophenol, isopropyl salicylate, and 2,4,5-trichlorophenol had no effect on the fate of their parent compounds. The results indicate that the hydrolysis metabolites could play an important role in the induction or inhibition of enhanced microbial degradation of some pesticides. Salicylic acid, a secondary hydrolysis metabolite of isofenphos, conditioned the soil for rapid degradation of isofenphos, whereas isopropyl salicylate, the primary hydrolysis metabolite, did not have any effect. This finding suggests that the microbes involved in enhanced degradation are sensitive to changes associated with the metabolism of the parent pesticide molecule. Also, it is evident that the secondary metabolites could play an important role in the environmental fate of pesticides.

A major variable that determines the microbial metabolism of soil-applied pesticides is the availability of the chemical to the microbial systems degrading it. A compound's mobility reflects its potential for availability in soil. The soil thin-layer chromatography technique was employed to assess the mobility of pesticides and their hydrolysis

metabolites in different soils. In general, metabolites such as p-nitrophenol, 2,4-dichlorophenol, and salicylic acid that were capable of conditioning the soil for enhanced degradation were mobile in most of the soils studied, indicating that their availability may be one of the factors influencing their potential to condition the soils for enhanced degradation. Isofenphos, a pesticide documented to undergo enhanced degradation, was not very mobile in soils. However, salicylic acid was mobile and its availability is important as it serves as a microbial substrate in the rapid degradation of isofenphos. The availability of metabolites may be more critical than that of parent compounds in influencing enhanced degradation. The susceptibility of pesticides to enhanced degradation only in specific parts of a state or country may be attributed to the influence of soil characteristics such as pH and organic matter on the availability of a chemical to microbes.

The toxicity of pesticides and their metabolites to soil microbes responsible for enhanced degradation seems to be a critical factor in the induction or inhibition of enhanced degradation. The relative microbial toxicities of pesticides and their metabolites were determined using the Microtox analyzer. The hydrolysis metabolites of some pesticides susceptible to enhanced degradation (isofenphos, diazinon, carbofuran) yielded higher EC_{50} values, reflecting their low toxicity to bacteria. However, 2,4-dichlorophenol that serves as an energy source to soil microbes recorded a low EC_{50} value. The toxicity of metabolites to *Photobacterium phosphoreum* may not correspond to toxicity to soil bacteria in all cases, but data indicate that susceptibility to enhanced

microbial degradation may be partially influenced by the toxicity or lack of toxicity of the metabolites to bacteria.

The results of the studies with hydrolysis metabolites show that the properties of some pesticide metabolites such as greater availability, low microbial toxicity, and nutritive value may favor enhanced degradation of their parent compounds in soils with populations of degrading microorganisms.

In addition to the focus on the role of hydrolysis metabolites in the development of enhanced degradation, the effect of short- and long-term history of manuring on the degradation of pesticides was studied. This study found no evidence of enhanced microbial degradation of pesticides in soils applied with hog manure for seven continuous years. The field in which the soils were collected had had a carbofuran failure, and the increased persistence of carbofuran in the manure-history soils suggest that manuring inhibited the development or expression of enhanced degradation. This increased persistence can be attributed to the value of manure as a nutritive alternate to pesticides as substrates and the changes in the pH and organic matter content of manure-amended soils.

Research to date has largely focused on the soil and microbial aspects of enhanced microbial degradation, and future research should aim at understanding the interaction between microbes and metabolites (primary as well as secondary) of pesticides. The information generated from such studies will benefit our understanding of the role of metabolites in persistence and degradation, phytotoxicity, and groundwater contamination potential of pesticides.

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